

AD \_\_\_\_\_

Award Number:

**W81XWH-07-1-0162**

TITLE:

**Pharmacological studies of NOP receptor agonists as novel analgesics**

PRINCIPAL INVESTIGATOR:

**Mei-Chuan Ko, Ph.D.**

CONTRACTING ORGANIZATION:

**University of Michigan  
Ann Arbor, MI 48109-5632**

REPORT DATE:

**May 2009**

TYPE OF REPORT:

**Annual report**

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

| REPORT DOCUMENTATION PAGE   |                  |                                 |                                      | Form Approved<br>OMB No. 0704-0188                        |  |
|---|------------------|---------------------------------|--------------------------------------|---|--|
| Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. <b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b>   |                  |                                 |                                      |   |  |
| 1. REPORT DATE (DD-MM-YYYY)<br>01-05-2009   |                  | 2. REPORT TYPE<br>Annual Report |                                      | 3. DATES COVERED (From - To)<br>15 Apr 2008 – 14 Apr 2009 |  |
| 4. TITLE AND SUBTITLE<br><br>Pharmacological Studies of NOP Receptor Agonists as Novel Analgesics   |                  |                                 |                                      | 5a. CONTRACT NUMBER                                       |  |
|   |                  |                                 |                                      | 5b. GRANT NUMBER<br>W81XWH-07-1-0162                      |  |
|   |                  |                                 |                                      | 5c. PROGRAM ELEMENT NUMBER                                |  |
| 6. AUTHOR(S)<br><br>Mei-Chuan Ko, Ph.D.   |                  |                                 |                                      | 5d. PROJECT NUMBER  |  |
|   |                  |                                 |                                      | 5e. TASK NUMBER   |  |
|   |                  |                                 |                                      | 5f. WORK UNIT NUMBER                                      |  |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)<br><br>University of Michigan<br>Ann Arbor, MI 48109-5632  |                  |                                 |                                      | 8. PERFORMING ORGANIZATION REPORT NUMBER                  |  |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)<br><br>U.S. Army Medical Research<br>and Material Command<br>Fort Detrick, Maryland 21702-5012  |                  |                                 |                                      | 10. SPONSOR/MONITOR'S ACRONYM(S)                          |  |
|   |                  |                                 |                                      | 11. SPONSOR/MONITOR'S REPORT NUMBER(S)                    |  |
| 12. DISTRIBUTION / AVAILABILITY STATEMENT<br><br>Approved for public release; distribution unlimited  |                  |                                 |                                      |   |  |
| 13. SUPPLEMENTARY NOTES   |                  |                                 |                                      |   |  |
| 14. ABSTRACT<br>The studies proposed in this project will test the hypotheses that in the non-human primate (1) the functions and behavioral effects of the NOP receptor are independent of classical opioid receptors, (2) activation of the NOP receptor produces strong antinociception without abuse liability, and (3) NOP receptor agonists possess a promising therapeutic profile as analgesics compared to mu opioids following repeated administration in primates. Several key findings have been obtained and some have been published. First, intrathecal administration of N/OFQ only produced antinociception in primates. The functional profiles of spinal NOP receptors are different between primates and rodents. Second, intrathecal administration of N/OFQ produced antinociception without eliciting itch/scratching responses, indicating that NOP receptor agonists represent a therapeutic target as spinal analgesics. Third, NOP receptor agonists produced antinociceptive effects comparable to clinically used mu opioids such as morphine and alfentanil in three different primate pain models, indicating that the analgesic effectiveness of NOP receptor agonists may be similar to that of mu opioid analgesics in humans. Finally, unlike mu opioids, NOP receptor agonists did not produce reinforcing effects, respiratory depressant, sedation, or itch/pruritic side effects, indicating that NOP receptor agonists may be a new generation of novel analgesics without abuse liability. |                  |                                 |                                      |   |  |
| 15. SUBJECT TERMS<br>Nociceptin/Orphanin FQ Peptide (NOP) Receptors   |                  |                                 |                                      |   |  |
| 16. SECURITY CLASSIFICATION OF:   |                  |                                 | 17. LIMITATION OF ABSTRACT<br><br>UU | 18. NUMBER OF PAGES<br><br>36                             | 19a. NAME OF RESPONSIBLE PERSON<br>USAMRMC |
| a. REPORT<br>U  | b. ABSTRACT<br>U | c. THIS PAGE<br>U               |                                      |   | 19b. TELEPHONE NUMBER (include area code)  |

## Table of Contents

|                                   | <u>Page</u> |
|-----------------------------------|-------------|
| Introduction.....                 | 1           |
| Body.....                         | 2           |
| Key Research Accomplishments..... | 13          |
| Reportable Outcomes.....          | 13          |
| Conclusion.....                   | 14          |
| References.....                   | 15          |
| Appendices.....                   | 16          |

## INTRODUCTION

Proposed studies intend to elucidate the potential antinociceptive effects of NOP receptor agonists in monkeys. Both intrathecal and systemic administration are common routes for delivery of analgesics in the clinic. Future studies characterizing and comparing the behavioral effects of intrathecal and systemic administration of OFQ/N and Ro 64-6198 in monkeys would provide a great deal of information for potential pain management in humans. In particular, the pharmacological profile and behavioral effects of NOP receptor agonists can be systematically compared with those of mu opioid receptor agonists in monkeys following acute and repeated administration, and they will make a notable advance in our understanding of pain and analgesia in relation to the fourth member of the opioid receptor family in primates. The studies proposed in this project will test the hypotheses that in the non-human primate (1) the functions and behavioral effects of the NOP receptor are independent of classical opioid receptors, (2) activation of the NOP receptor produces strong antinociception without abuse liability, and (3) NOP receptor agonists possess a promising therapeutic profile as analgesics compared to mu opioids following repeated administration in primates.

## BODY

### TASK 1.

#### Extensive evaluation of the behavioral effects of intrathecally administered N/OFQ in non-human primates.

(a) Study behavioral effects of ultra-low doses of intrathecal N/OFQ over a wide dose range using a warm water tail withdrawal assay and behavioral observations.

**Table 1.** Behavioral responses of intrathecal administration of N/OFQ over a wide range of ultra-low doses as compared to a single dose of DAMGO and substance P.

| Compound/Dose      | Warm water tail-withdrawal latency (sec) <sup>a</sup> |             | Itch/Scratching <sup>b</sup> |
|--------------------|---|-------------|------------------------------|
|                    | 46°C  | 50°C        | Number/15 min                |
| <b>N/OFQ</b>       |   |             |                              |
| 0 (vehicle)        | 20 ± 0 <sup>c</sup>                                   | 1.6 ± 0.1   | 50.0 ± 12.9                  |
| 1 fmol             | 20 ± 0  | 1.7 ± 0.1   | 33.8 ± 9.9                   |
| 10 fmol            | 20 ± 0  | 1.6 ± 0.2   | 49.3 ± 15.7                  |
| 100 fmol           | 20 ± 0  | 1.4 ± 0.2   | 44.3 ± 14.0                  |
| 1 pmol             | 20 ± 0  | 1.7 ± 0.1   | 57.2 ± 15.3                  |
| 10 pmol            | 20 ± 0  | 1.8 ± 0.1   | 57.5 ± 10.2                  |
| 100 pmol           | 20 ± 0  | 1.9 ± 0.2   | 41.0 ± 15.1                  |
| 1 nmol             | 20 ± 0  | 1.6 ± 0.2   | 35.2 ± 7.1                   |
| <b>Substance P</b> |   |             |                              |
| 100 nmol           | 4.9 ± 1.4*  | 1.2 ± 0.1   | 48.5 ± 8.6                   |
| <b>DAMGO</b>       |   |             |                              |
| 10 nmol            | 20 ± 0  | 16.8 ± 2.1* | 910.5 ± 103.9*               |

a) The latency was measured at 15 min after intrathecal administration of test compound.

b) The scratching number was scored between 15<sup>th</sup> and 30<sup>th</sup> min after intrathecal administration of test compound.

c) Each value represents mean ± S.E.M. (n=6).

\*) The asterisk represents a significant difference from the vehicle condition (p<0.05).

Table 1 shows that intrathecal administration of N/OFQ over a wide dose range from 1 fmol to 1 nmol did not produce hyperalgesia, scratching, or any pain-like behavioral responses in monkeys. Ultra-low doses of intrathecal N/OFQ (i.e., fmol) produced pain-like behavior manifested by scratching, biting, and licking behaviors in mice (Sakurada et al., 1999). The pharmacological profile of intrathecal N/OFQ is clearly different between rodents and primates (Ko et al., 2006).

In addition, intrathecal N/OFQ at doses between 10 nmol and 1  $\mu$ mol dose-dependently produced antinociceptive effects against a noxious stimulus at different intensities. Combined administration of intrathecal N/OFQ and morphine significantly potentiated morphine-induced antinociception without inhibiting morphine-induced itch/scratching responses. Although these experiments were time-consuming and labor-intensive, these results provide a unique functional profile of intrathecal N/OFQ over a wide dose range in primates. Overall, intrathecal N/OFQ produced thermal antinociception without anti-morphine actions or eliciting itch/scratching responses, indicating that N/OFQ or NOP receptor agonists may represent a promising target as spinal analgesics.

Findings relevant to Task 1 have been published in the Journal of Pain, the official journal of *American Pain Society* ([Ko MC & Naughton NN \(2009\) Antinociceptive effects of nociception/orphanin FQ administered intrathecally in monkeys. Journal of Pain 10\(5\):509-516](#), see Appendices for other details).

## **TASK 2.**

### **Comparison of effectiveness of systemically administered Ro 64-6198 in different experimental pain models in non-human primates.**

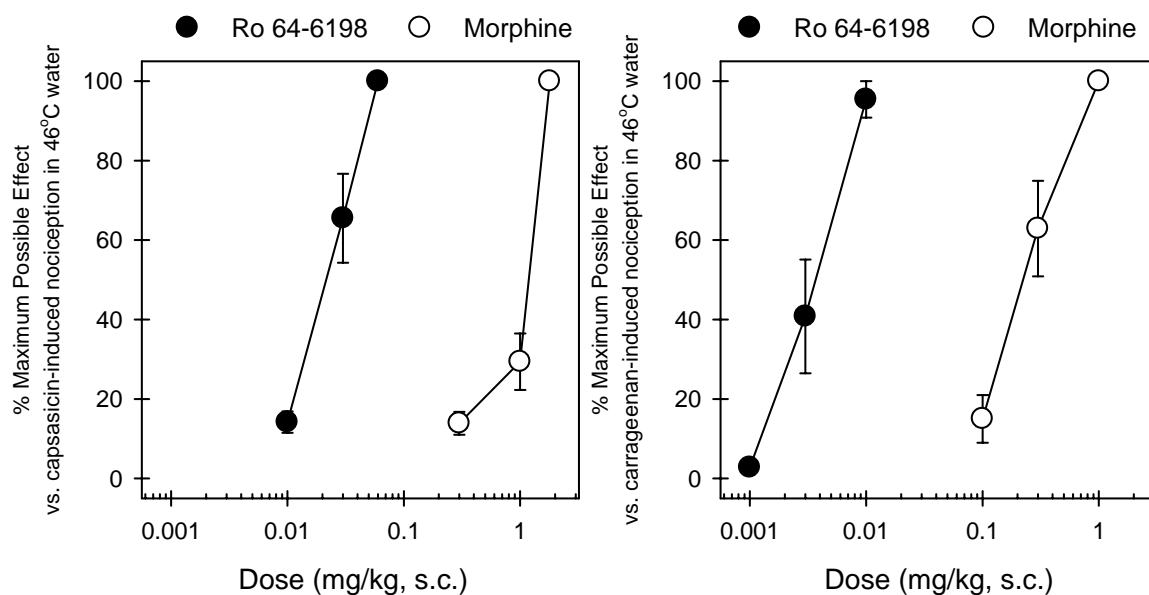
- (a) Determine the doses of systemic Ro 64-6198, a non-peptidic NOP receptor-selective agonist, that produce antinociception in monkeys using a warm water 50°C tail withdrawal assay.

This experiment has been conducted. Systemic administration of Ro 64-6198 (0.001-0.03 mg/kg), a NOP receptor-selective agonist, dose-dependently produced antinociceptive effects against a noxious stimulus, 50°C water. Systemic Ro 64-6198

0.03 mg/kg produced full antinociception under this context. The warm water tail-withdrawal assay has been widely used to determine the antinociceptive effects of the test compound in monkeys (Butelman et al., 1993; Ko et al., 1998a). Previous studies have shown that systemic morphine 3 mg/kg produced full antinociception measured by this procedure (Butelman et al., 1996; Lee et al., 2007).

- (b) Compare the antinociceptive effects of systemic Ro 64-6198 with those of systemic morphine in capsaicin-induced allodynia and carrageenan-induced hyperalgesia in the same monkeys.

This experiment has been conducted. **Figure 1** shows the antinociceptive effectiveness and potency of morphine and Ro 64-6198 against two different nociceptive assays. Both Ro 64-6198 and morphine are effective in producing antinociception against two different noxious stimuli. More importantly, Ro 64-6198 is more potent (~50-100 fold) than morphine to produce anti-allodynic/anti-hyperalgesic effects under this context.



**Figure 1.** Antinociceptive effects of Ro 64-6198 and morphine against capsaicin- and carrageenan-induced allodynia/hyperalgesia in 46 °C water.

Both capsaicin- and carrageenan-induced pain models have been established in monkeys to determine and compare the effectiveness of clinically used analgesics and experimental compounds (Ko et al., 1998b; Ko and Lee, 2002; Butelman et al., 2004). In particular, a capsaicin-based pain model is practical and valuable on many levels. Capsaicin is a natural irritant found in hot-chili peppers that evokes pain sensation by activating at the TRPV1. TRPV1 and the up-regulation of its expression have been strongly implicated in the integration and transduction of a variety of pain signaling including tissue-injury induced thermal hyperalgesia, diabetic neuropathy, and neurogenic inflammatory response associated with many disease states (Szallasi et al., 2007; Knotkova et al., 2008). Furthermore, capsaicin-induced allodynia has been previously utilized as a pain model in both monkeys (Ko et al., 1998b; Butelman et al., 2004) and humans (Park et al., 1995; Eisenach et al., 1997) to study experimental compounds as analgesics. Considering the variety of pain modalities capsaicin-sensitive fibers are linked to, the ability to attenuate capsaicin-induced allodynia would suggest a prominent clinical value of NOP receptor agonists.

Part of findings relevant to Task 2 has been published in *Neuropsychopharmacology*, the official journal of the *American College of Neuropsychopharmacology* (Ko MC et al. (2009) Behavioral effects of a synthetic agonist selective for nociception/orphanin FQ peptide receptors in monkeys. *Neuropsychopharmacology*, advance online publication, DOI: 10.1038/npp.2009.33, see Appendices for other details).

### **TASK 3.**

#### **Clarification of the receptor selectivity and site of actions of NOP receptor agonists by conducting receptor antagonist studies *in vivo*.**

- (a) Determine the *in vivo* apparent  $pA_2$  value of J-113397, a non-peptidic NOP receptor-selective antagonist, against systemic Ro 64-6198-induced antinociception in monkeys.

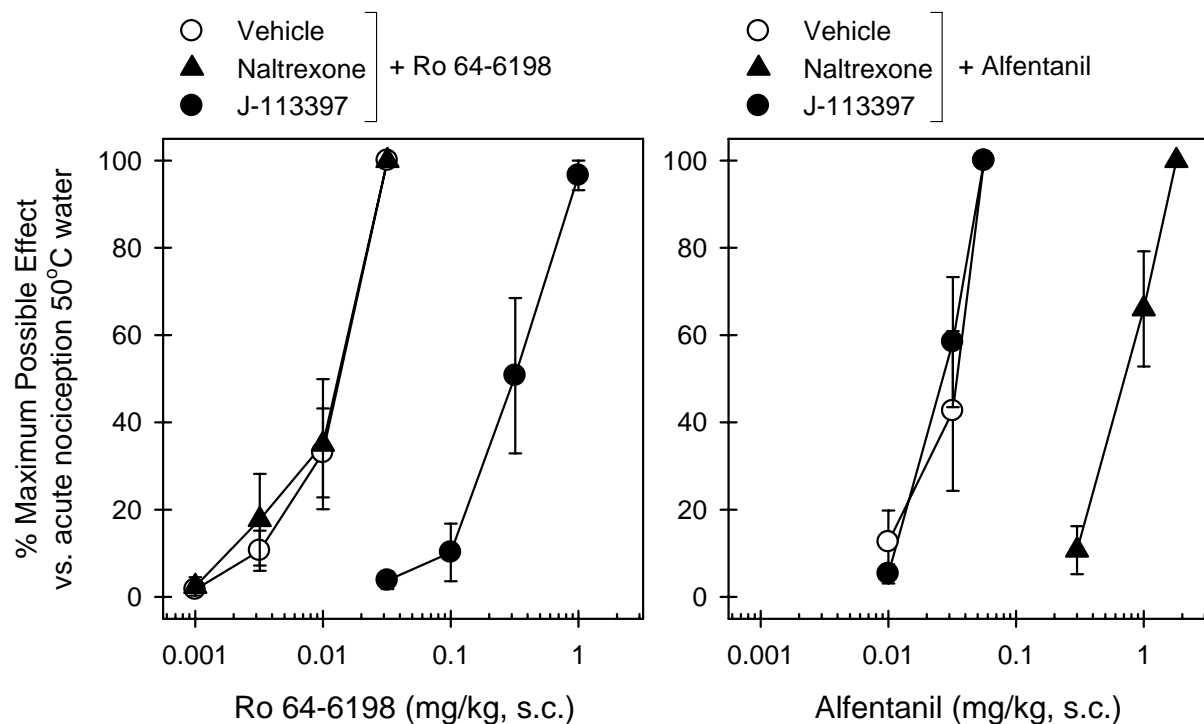
This experiment has been conducted. Pretreatment with J-113397 dose-dependently produced rightward shifts of the dose response curve of Ro 64-6198-induced antinociception. These dose-dependent antagonist effects of J-113397 were



graphed in a Schild plot with values derived from individual dose ratios for each subject. The mean  $pA_2$  value of J-113397 was 7.98 (7.85-8.11) with a slope of -1. The doses of J-113397 alone did not change the thermal threshold of monkeys (i.e., no changes in the tail withdrawal latencies in 42, 46, or 50°C water).

(b) Cross-examine the antagonist potency of naltrexone, an opioid receptor antagonist, on Ro 64-6198-induced antinociception and the antagonist potency of J-113397 on morphine-induced antinociception.

This experiment has been conducted. **Figure 2** compares the antagonist effects of naltrexone and J-113397 on the antinociceptive effects produced by s.c. Ro 64-6198 and alfentanil.



**Figure 2.** Effects of mu opioid receptor and NOP receptor antagonists on alfentanil- and Ro 64-6198-induced antinociceptive effects in monkeys.

The left panel shows that a single dose (0.1 mg/kg) of J-113397 produced a large rightward shift of the dose response curve of Ro 64-6198-induced antinociception. The mean J-113397  $pK_B$  value was 8.02 (7.78-8.26) under this condition. Naltrexone 0.03 mg/kg failed to block Ro 64-6198-induced antinociception; the ED50 value of Ro 64-6198 dose response for vehicle pretreatment (0.012 mg/kg) was similar to that for naltrexone pretreatment (0.013 mg/kg). In contrast, the right panel shows that a single dose of naltrexone 0.03 mg/kg produced a large rightward shift of the dose response curve of alfentanil-induced antinociception. The mean naltrexone  $pK_B$  value was 8.44 (8.18-8.70) under this condition. J-113397 0.1 mg/kg failed to block alfentanil-induced antinociception; the ED50 value of alfentanil dose response for vehicle pretreatment (0.031 mg/kg) was similar to that for J-113397 pretreatment (0.026 mg/kg).

(c) Compare the antagonist potency of intrathecal versus subcutaneous J-113397 on systemic Ro 64-6198-induced antinociception.

This experiment has been conducted. Pretreatment with a single dose 0.01 mg/kg of subcutaneous J-113397 produced approximately a 30-fold rightward shift of the dose-response curve of Ro 64-6198-induced antinociception. In contrast, pretreatment with a single dose 0.001 mg (i.e., 100-fold less than total amount 0.1 mg afforded by subcutaneous J-113397 0.01 mg/kg in monkeys with averaged body weight of 10 kg) of intrathecal J-113397 produced approximately a 25-fold rightward shift of the dose response curve of Ro 64-6198-induced antinociception.

Taken together, these findings showed that systemic Ro 64-6198 alone produced antinociceptive effects which could be blocked dose-dependently by J-113397, a selective NOP receptor antagonist. In vivo apparent  $pA_2$  analysis was used because this quantitative procedure offers a powerful approach to establish receptor-mediated drug effects (Arunlakshana and Schild, 1959; Tallarida et al., 1979). J-113397 dose-dependently produced parallel rightward shifts of the dose response curve of Ro 64-6198-induced antinociception, indicating that the agonist and antagonist compete for the same NOP receptors in a reversible manner. More importantly, cross-examination of both antagonists against different agonists demonstrated that both alfentanil- and Ro 64-6198-induced antinociceptive effects were mediated by mu opioid receptors and

NOP receptors, respectively. In addition, an ultra-small dose of J-113397 produced a similar magnitude of the rightward shift of the dose response curve of Ro 64-6198 antinociception compared to the systemic active dose of J-113397. These experiments provide a pharmacological basis for the role of spinal NOP receptors in Ro 64-6198-induced antinociception and indicate that antinociceptive effects of opioid analgesics can be produced by two independent opioid receptor mechanisms in monkeys.

Part of findings relevant to Task 3 has also been published in Neuropsychopharmacology (Ko MC et al. (2009) Behavioral effects of a synthetic agonist selective for nociception/orphanin FQ peptide receptors in monkeys. Neuropsychopharmacology, advance online publication, DOI: 10.1038/npp.2009.33, see Appendices for other details).

#### **TASK 4.**

##### **Evaluation of potential abuse liability of NOP receptor agonists using the self-administration assay.**

(a) Determine and compare reinforcing effects of Ro 64-6198 with those of the mu opioid agonist fentanyl, the psychomotor stimulant cocaine, and the barbiturate anesthetic methohexital in the monkey intravenous self-administration assay to assess whether NOP receptor agonists possess abuse liability.

This experiment has been conducted. Response rates (responses/sec) for saline, alfentanil, and Ro 64-6198 across a dose range of 0.03 – 30 µg/kg/inj were assessed. To aggregate data across all subjects, mean response rates engendered by each dose of each drug were averaged. Under this multiple component schedule, contingent saline infusions engendered very low response rates (less than 0.3 responses/sec). All animals self-administered alfentanil within the dose range tested, generating a biphasic dose-effect curve characteristic of intravenous drug self-administration. In contrast, Ro 64-6198 did not maintain high rates of responding at any of the doses tested, resulting in a flat dose-effect curve indicative of a compound without reinforcing effects under the present conditions. Likewise, Ro 64-6198 did not maintain high rates of responding at doses tested, but all subjects self-administered cocaine, under the same schedule.

(b) Assess the effects of Ro 64-6198 pretreatment on remifentanil- and cocaine-maintained self-administration behavior.

This experiment has been conducted. Pretreatment with an antinociceptive dose 0.03 mg/kg of Ro 64-6198 did not significantly attenuate the monkey's self-administration responses maintained by either cocaine (0.01 mg/kg/injection) or remifentanil (0.1 µg/kg/injection) under a single test session.

Taken together, these findings showed lack of reinforcing effects of Ro 64-6198 in alfentanil-, cocaine-, and methohexital-maintained monkeys. The presence of a behavioral effect (i.e., antinociception at 10-30 µg/kg) in the absence of any indication of a reinforcing effect indicates that we have tested sufficiently large doses for potential reinforcing effects. For example, the antinociceptive doses of intravenous alfentanil were 10-30 µg/kg (Ko et al., 2002), but the doses of alfentanil producing reinforcing effects were 0.1-1 µg/kg (i.e., a 30-100 fold difference) (Winger et al., 1992; Ko et al., 2002). Lack of reinforcing effects by Ro 64-6198 might be expected because several studies have shown that activation of NOP receptors inhibited dopamine release in the striatum and supported the notion that NOP receptor agonists do not have reinforcing or aversive properties of their own (Murphy and Maidment, 1999; Flau et al., 2002). The relation between NOP receptors and dopamine release was also supported by the findings that pretreatment with Ro 64-6198 did not attenuate opioids such as remifentanil- or cocaine-mediated reinforcing effects in monkeys.

Part of findings relevant to Task 4 has also been published in Neuropsychopharmacology ([Ko MC et al. \(2009\) Behavioral effects of a synthetic agonist selective for nociception/orphanin FQ peptide receptors in monkeys. Neuropsychopharmacology, advance online publication, DOI: 10.1038/npp.2009.33, see Appendices for other details\).](#)

## **TASK 5.**

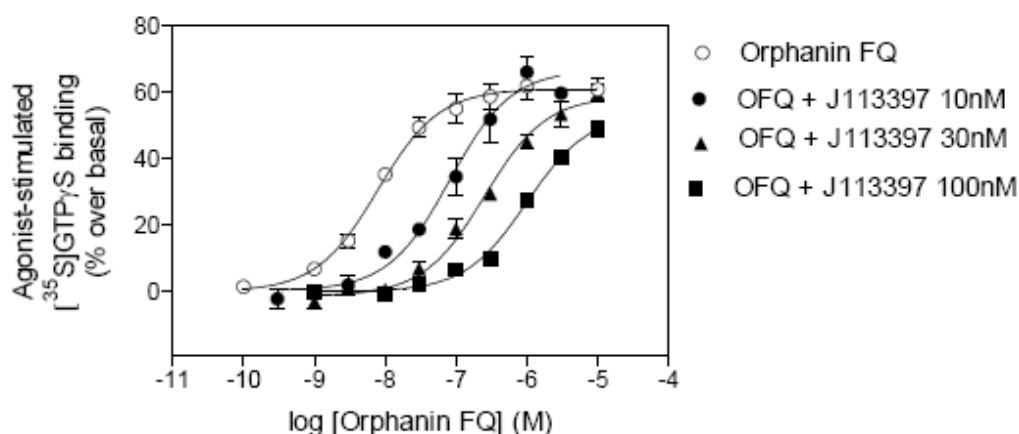
### **Determination of the receptor selectivity and functional efficacy of NOP receptors at the cellular level.**

(a) Characterize the density of NOP receptors in membranes of cortex, thalamus, and spinal cord of monkeys by using a receptor binding assay.

This experiment has been initiated. We anticipate informative and conclusive data will be available in the near future.

- (b) Compare the potency and magnitude of concentration-responses curves of N/OFQ- and Ro 64-6198-stimulated [ $^{35}$ S]GTP $\gamma$ S binding in membranes of cortex, thalamus, and spinal cord of monkeys.

This experiment has also been initiated. **Figure 3** shows the antagonist effects of J-113397 on N/OFQ-stimulated [ $^{35}$ S]GTP $\gamma$ S binding in cortical membranes of a single monkey. More data will be collected to clarify the receptor selectivity and functional efficacy of NOP receptors at the cellular level.



**Figure 3.** Antagonist effects of J-113397 on N/OFQ-stimulated [ $^{35}$ S]GTP $\gamma$ S binding in cortical membranes of a single monkey. Open circles represent a concentration-response curve of N/OFQ. Filled symbols represent concentration-response curves of N/OFQ in the present of different concentrations of J-113397.

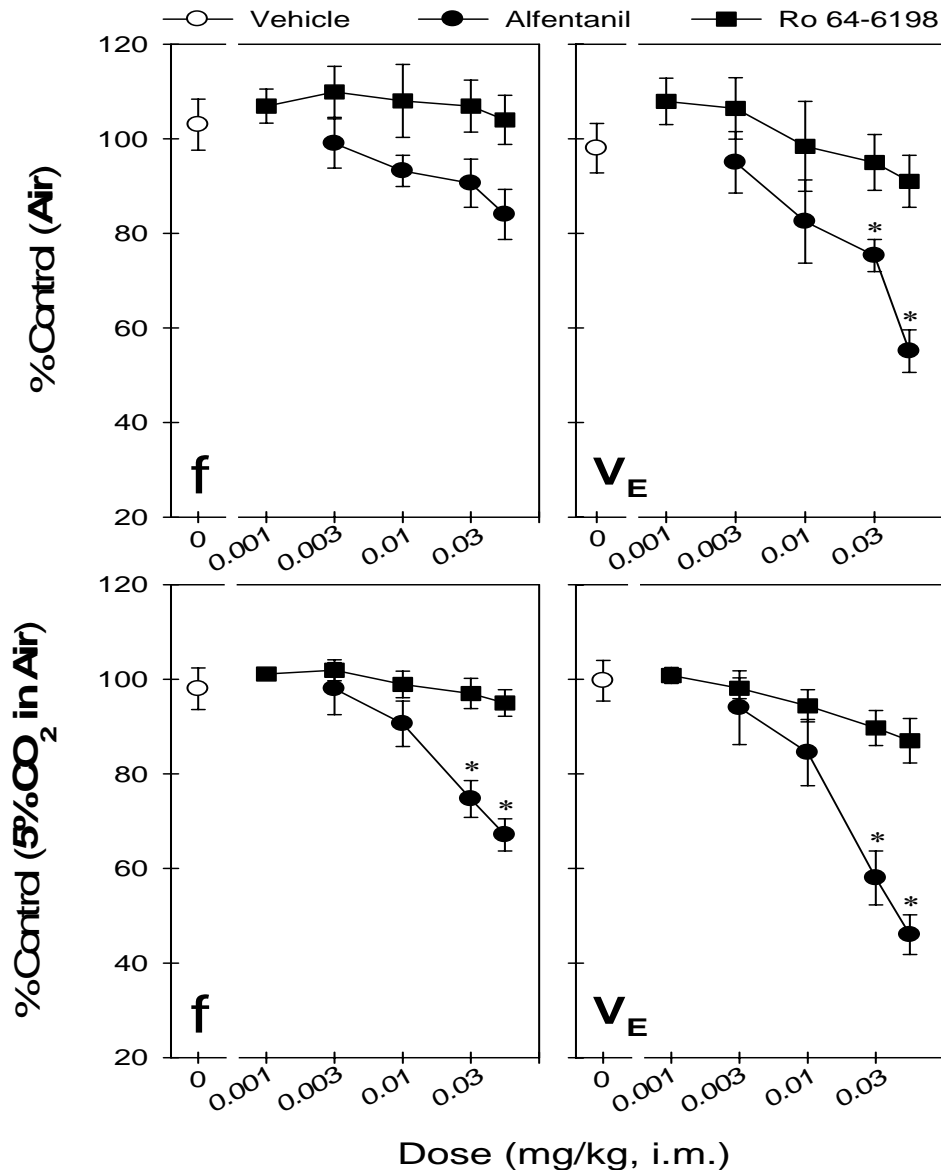
#### **TASK 6.**

#### **Evaluation of the behavioral profile and safety margin of the NOP receptor agonist in non-human primates.**

- (a) Determine whether the dose equal to or larger than antinociceptive doses of

systemic Ro 64-6198 produce side effects such as respiratory depression, sedation, and convulsions and whether J-113397 can reverse the side effects of Ro 64-6198 in monkeys.

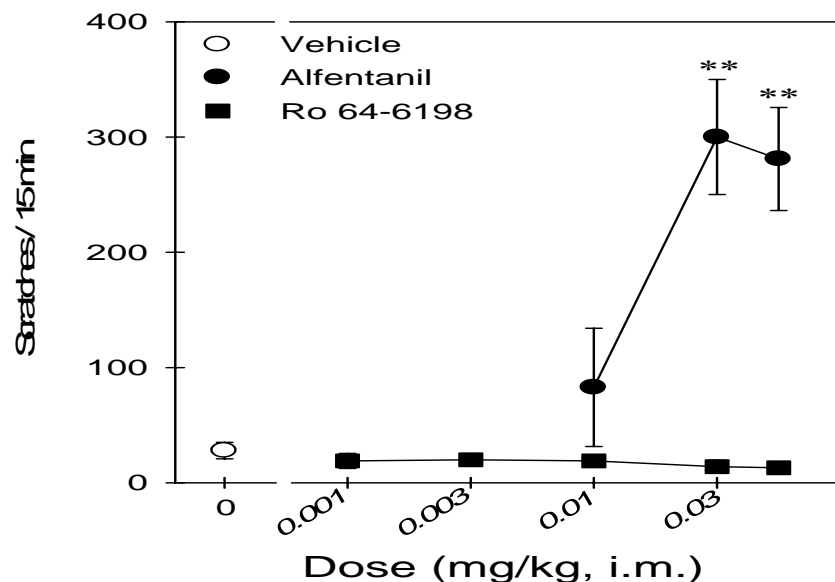
This experiment has been conducted. **Figure 4** compares the dose-response curves of Ro 64-6198 and alfentanil for the changes of respiratory parameters  $f$  and  $V_E$  during breathing air or a mixture of 5%  $CO_2$  in air. Alfentanil dose-dependently



**Figure 4.** Comparison of the dose-response curves for respiratory depressant effects produced by intramuscular administration of alfentanil and Ro 64-6198.

decreased *f* and VE responses, but Ro 64-6198 did not significantly decrease the respiratory function, compared with the vehicle condition in monkeys under both breathing cycles. More importantly, a dose (0.06 mg/kg) larger than the antinociceptive dose (0.01-0.03 mg/kg) of Ro 64-6198 did not significantly decrease the respiratory parameters under this context.

In addition, **Figure 5** compares the itch/scratching responses of alfentanil and Ro 64-6198 after intramuscular administration. Alfentanil dose-dependently elicited scratching responses. In contrast, Ro 64-6198 did not increase scratching responses compared with the vehicle condition in the same monkeys. These doses of Ro 64-6198 (0.001-0.06 mg/kg) did not produce any observable sedation in monkeys.



**Figure 5.** Comparison of the dose-response curves for itch/scratching effects produced by intramuscular administration of alfentanil and Ro 64-6198.

Part of findings relevant to Task 6 has also been published in *Neuropsychopharmacology* (Ko MC et al. (2009) Behavioral effects of a synthetic agonist selective for nociception/orphanin FQ peptide receptors in monkeys.

[Neuropsychopharmacology](https://doi.org/10.1038/npp.2009.33), advance online publication, DOI: 10.1038/npp.2009.33, see Appendices for other details).

## **KEY RESEARCH ACCOMPLISHMENTS**

These findings indicate that -

- Intrathecal administration of N/OFQ only produced antinociception in primates. The functional profiles of spinal NOP receptors are different between primates and rodents.
- Intrathecal administration of N/OFQ produced antinociception without eliciting itch/scratching responses, indicating that N/OFQ or other NOP receptor agonists represent a therapeutic target as spinal analgesics.
- NOP receptor agonists produced antinociceptive effects comparable to clinically used mu opioids such as morphine and alfentanil in three different primate pain models, indicating that the analgesic effectiveness of NOP receptor agonists may be similar to that of mu opioid analgesics in humans.
- Unlike mu opioids, NOP receptor agonists did not produce reinforcing effects, respiratory depressant, sedation, or itch/pruritic side effects, indicating that NOP receptor agonists may be a new generation of novel analgesics without abuse liability.

## **REPORTABLE OUTCOMES**

1. Ko MC and Naughton NN (2009)

Antinociceptive effects of nociception/orphanin FQ administered intrathecally in monkeys.

Journal of Pain, Vol. 10, No. 5, pp 509-516. (see Appendices for other details).



2. Ko MC, Woods JH, Fantegrossi WE, Galuska CM, Wichmann J, Prinssen EP (2009) Behavioral effects of a synthetic agonist selective for nociception/orphanin FQ peptide receptors in monkeys. *Neuropsychopharmacology*, advance online publication, DOI: 10.1038/npp.2009.33, see Appendices for other details).

## CONCLUSION

These experiments conducted so far demonstrated two important points. The first point is in the field of using spinal opioid analgesics. Spinal administration of mu opioid analgesics is an important method for pain management in the past few decades. However, itch/pruritus is the most common side effects derived from spinal opioids. Intrathecal administration of morphine dose-dependently produces antinociception with simultaneous scratching responses in monkeys, and this observation parallels closely with the functional profile of spinal morphine in humans. Using the monkey model, NOP receptor agonists only produced antinociceptive effects without eliciting itch/scratching responses. Such findings strongly indicate that NOP receptor agonists represent a therapeutic target as spinal analgesics.

The second point is in the research and development of novel opioid analgesics. As a recent review (Corbett et al., 2006) pointed out, much effort aimed at developing powerful analgesics without the side effects associated with mu opioids. Using the monkey model, NOP receptor agonists display a very different pharmacological profile compared to rodents. Like mu opioids, Ro 64-6198 produced full antinociceptive effects in three primate pain models. Unlike mu opioids, Ro 64-6198 did not producing reinforcing effects, respiratory depression, or itch/pruritic effects, indicating that NOP receptor agonists may be a new generation of novel analgesics without abuse liability. Such a promising pharmacological profile warrants additional monkey studies to investigate effects of other NOP receptor agonists and initiation of clinical trials of NOP receptor agonists in humans.

## REFERENCES

- Arunlakshana O, Schild HO (1959). Some quantitative uses of drug antagonists. *Br J Pharmacol* **14**: 48-58.
- Butelman ER, Negus SS, Ai Y, de Costa BR, Woods JH (1993). Kappa opioid antagonist effects of systemically administered nor-binaltorphimine in a thermal antinociception assay in rhesus monkeys. *J Pharmacol Exp Ther* **267**: 1269-1276.
- Butelman ER, Lewis JW, Woods JH (1996) Methoclocinnamox: agonist and antagonist effects of a novel long-lasting opioid in rhesus monkeys. *J Pharmacol Exp Ther* **279**: 934-938.
- Butelman ER, Harris TJ, Kreek MJ (2004). Antiallodynic effects of loperamide and fentanyl against topical capsaicin-induced allodynia in unanesthetized primates. *J Pharmacol Exp Ther* **311**: 155-163.
- Corbett AD, Henderson G, McKnight AT, Paterson SJ (2006) 75 years of opioid research: the exciting but vain quest for the Holy Grail. *Br J Pharmacol* **147**: S153-S162.
- Eisenach JC, Hood DD, Curry R, Tong C (1997). Alfentanil, but not amitriptyline, reduces pain, hyperalgesia, and allodynia from intradermal injection of capsaicin in humans. *Anesthesiology* **86**: 1279-1287.
- Flau K, Redmer A, Liedtke S, Kathmann M, Schlicker E (2002). Inhibition of striatal and retinal dopamine release via nociceptin/orphanin FQ receptors. *Br J Pharmacol* **137**:1355-1361.
- Knotkova H, Pappagallo M, Szallasi A (2008) Capsaicin (TRPV1 Agonist) therapy for pain relief: farewell or revival? *Clin J Pain* **24**: 142-154.
- Ko MC, Butelman ER, Traynor JR, Woods JH (1998a). Differentiation of kappa opioid agonist-induced antinociception by naltrexone apparent pA<sub>2</sub> analysis in rhesus monkeys. *J Pharmacol Exp Ther* **285**: 518-526.
- Ko MC, Butelman ER, Woods JH (1998b). The role of peripheral mu opioid receptors in the modulation of capsaicin-induced thermal nociception in rhesus monkeys. *J Pharmacol Exp Ther* **286**: 150-156.
- Ko MC, Lee H (2002) An experimental model of inflammatory pain in monkeys:

- comparison of antinociceptive effects of opioids and NSAIDs against carrageenan-induced thermal hyperalgesia. The 10<sup>th</sup> World Congress on Pain (Abstract), p. 136, International Association for the Study of Pain (IASP) Press, Seattle, WA, USA.
- Ko MC, Turner J, Hursh S, Woods JH, Winger G (2002). Relative reinforcing effects of three opioids with different durations of action. *J Pharmacol Exp Ther* **301**: 698-704.
- Ko MCH, Wei H, Woods JH, Kennedy RT (2006) Effects of intrathecally administered nociceptin/orphanin FQ in monkeys: Behavioral and mass spectrometric studies. *J Pharmacol Exp Ther* 318:1257-1264.
- Lee H, Naughton NN, Woods JH, Ko MC (2007) Effects of butorphanol on morphine-induced itch and analgesia in primates. *Anesthesiology* **107**: 478-485.
- Murphy NP, Maidment NT (1999). Orphanin FQ/nociceptin modulation of mesolimbic dopamine transmission determined by microdialysis. *J Neurochem* **73**: 179-186.
- Park KM, Max MB, Robinovitz E, Gracely RH, Bennett GJ (1995). Effects of intravenous ketamine, alfentanil, or placebo on pain, pinprick hyperalgesia, and allodynia produced by intradermal capsaicin in human subjects. *Pain* **63**: 163-172.
- Sakurada T, Katsuyama S, Sakurada S, Inoue M, Tan-No K, Kisara K, Sakurada C, Ueda H and Sasaki J (1999). Nociceptin-induced scratching, biting and licking in mice: involvement of spinal NK1 receptors. *Br J Pharmacol* 127:1712-1718.
- Szallasi A, Cortright DN, Blum CA, Eid SR (2007). The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof of concept. *Nat Rev Drug Discov* **6**: 357-372.
- Tallarida RJ, Cowan A, Adler MW (1979). pA<sub>2</sub> and receptor differentiation: A statistical analysis of competitive antagonism. *Life Sci* **25**: 637-654.
- Winger G, Skjoldager P, Woods JH (1992). Effects of buprenorphine and other opioid agonists and antagonists on alfentanil- and cocaine-reinforced responding in rhesus monkeys. *J Pharmacol Exp Ther* **261**: 311-317.

## **APPENDICES** (see attachment)

## Antinociceptive Effects of Nociceptin/Orphanin FQ Administered Intrathecally in Monkeys

Mei-Chuan Ko<sup>\*,†</sup> and Norah N. Naughton<sup>‡</sup>

<sup>\*</sup>Department of Pharmacology.

<sup>†</sup>Departments of Anesthesiology and Obstetrics and Gynecology, University of Michigan Medical School, Ann Arbor, Michigan.

<sup>‡</sup>Department of Psychology and Institute of Neuroscience, National Cheng-Chi University, Taipei, Taiwan.

**Abstract:** Nociceptin/orphanin FQ (N/OFQ) is the endogenous peptide for the NOP receptors. Depending on the doses, intrathecal administration of N/OFQ has dual actions (ie, hyperalgesia and antinociception) in rodents. However, the pharmacological profile of intrathecal N/OFQ is not fully known in primates. The aim of this study was to investigate behavioral effects of intrathecal N/OFQ over a wide dose range and to compare its effects with ligands known to produce hyperalgesia or antinociception in monkeys. Intrathecal N/OFQ from 1 fmol to 1 nmol did not produce any hyperalgesic or scratching responses. In contrast, intrathecal substance P 100 nmol produced hyperalgesia, and intrathecal DAMGO 10 nmol produced antinociception. At the dose range between 10 nmol and 1  $\mu$ mol, intrathecal N/OFQ dose-dependently produced thermal antinociception against a noxious stimulus in 2 intensities. More importantly, N/OFQ in combined with intrathecal morphine dose-dependently potentiated morphine-induced antinociception without inhibiting morphine-induced itch/scratching. Taken together, this study is the first to provide a unique functional profile of intrathecal N/OFQ over a wide dose range in primates. Intrathecal N/OFQ produces thermal antinociception without anti-morphine actions or scratching responses, indicating that N/OFQ or NOP receptor agonists represent a promising target as spinal analgesics.

**Perspective:** Intrathecal administration of N/OFQ only produced thermal antinociception, not hyperalgesia, in monkeys. In addition, intrathecal N/OFQ does not have anti-morphine actions or itch/scratching responses. This study strongly supports the therapeutic potential of N/OFQ or NOP receptor agonists as spinal analgesics for clinical trials.

© 2009 by the American Pain Society

**Key words:** Spinal cord, analgesia, NOP receptors, substance P, thermal hyperalgesia.

Spinal administration of  $\mu$ -opioid receptor agonists is an important method for pain management, and it is widely used for obstetric analgesia.<sup>8,10</sup> However, itch/pruritus is the most common side effect derived from spinal opioids, and it reduces the value of pain relief afforded by spinal opioids.<sup>8,14</sup> Previously, we have established an experimental model of spinal opioid-induced itch/scratching in monkeys.<sup>18,21</sup> Intrathecal administration of morphine dose-dependently produces

antinociception with simultaneous scratching responses in monkeys,<sup>18</sup> and this observation parallels closely with the behavioral effects of spinal morphine in humans.<sup>1,34</sup> This experimental model using the intrathecal route for drug delivery in primates provides a valuable tool for identifying a novel, viable target as spinal analgesics.

Interestingly, a recent study found that intrathecal administration of an endogenous peptide, nociceptin/orphanin FQ (N/OFQ),<sup>28,36</sup> in the dose range of nanomoles produced antinociceptive effects without itch/scratching responses in monkeys.<sup>22</sup> Such naltrexone-insensitive effects could be blocked by the selective N/OFQ peptide receptor (NOP) antagonist J-113397 indicating that activation of spinal NOP receptors may be a promising target for spinal analgesia.<sup>22,24</sup> However, ultra low doses of N/OFQ administered intrathecally at the dose range of femtomoles produced spontaneous agitation and pain manifested by biting, scratching, and licking behavioral

Received September 11, 2008; Revised November 4, 2008; Accepted November 11, 2008.

Supported by United States Department of Defense, Peer Reviewed Medical Research Program, Grant No. W81XWH-07-1-0162. Address reprint requests to Dr M.C. Ko, Department of Pharmacology, University of Michigan Medical School, 1301 MSRB III, Ann Arbor, MI 48109-5632. E-mail: [mko@umich.edu](mailto:mko@umich.edu).

1526-5900/\$36.00

© 2009 by the American Pain Society

doi:10.1016/j.jpain.2008.11.006

responses in mice, suggesting that spinal N/OFQ has biphasic actions in rodents.<sup>15,40</sup> Anatomical studies indicated that species differences may exist in the distribution of N/OFQ and NOP receptors.<sup>2,4</sup> Nevertheless, most studies report that there is a high expression of N/OFQ and NOP receptors in the spinal cord of both rodents and humans.<sup>31,44</sup> It is worth investigating whether spinal N/OFQ has both antinociceptive and pronociceptive/hyperalgesic actions and further characterizing the physiological functions of spinal N/OFQ in primates.

Therefore, the aim of this study was to extensively investigate and directly compare the behavioral effects of intrathecally administered N/OFQ over a wide dose range in monkeys. As noted, rodent studies have shown that intrathecal DAMGO and substance P produced antinociceptive and pronociceptive effects, respectively.<sup>29,30,41</sup> By using both behavioral end points (ie, antinociception/hyperalgesia and scratching responses), effects of intrathecal DAMGO and substance P were compared with those of intrathecal N/OFQ. Antinociceptive effects of intrathecal N/OFQ were further studied against a noxious stimulus in 2 intensities. In addition, the potential interaction between intrathecal N/OFQ and morphine was determined to explore whether N/OFQ modulated intrathecal morphine-induced antinociception and scratching responses.

## Materials and Methods

### Subjects

Eighteen adult intact male and female rhesus monkeys (*Macaca mulatta*) with body weights ranging between 6.7 and 12.2 kg were used. The monkeys were housed individually with free access to water and were fed approximately 25 to 30 biscuits (Purina Monkey Chow; Ralston Purina, St. Louis, MO) and fresh fruit daily. No monkey had exposure to any opioid 1 month before the present study. The monkeys were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care. The studies were conducted in accordance with the University Committee on the Use and Care of Animals in the University of Michigan (Ann Arbor, MI) and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health (Bethesda, MD).

### Procedures

#### Nociceptive Responses

The warm water tail-withdrawal assay was used to evaluate thermal antinociceptive or hyperalgesic effects of the test compound.<sup>19,22</sup> Briefly, monkeys were seated in primate restraint chairs, and the lower part of their shaved tails (approximately 15 cm) were immersed in a thermal flask containing water maintained at either 42°, 46°, 50°, or 54°C. Tail-withdrawal latencies were measured using a computerized timer by an experimenter who did not know dosing conditions. In each test session, monkeys were evaluated once with 4 temperatures given in a random order. If the monkeys

did not remove their tails within 20 seconds (cutoff), the flask was removed and a maximum time of 20 seconds was recorded. Test sessions began with determining a control value at each temperature. Subsequent tail-withdrawal latencies were determined at multiple time points after intrathecal administration.

#### Itch/Scratching Responses

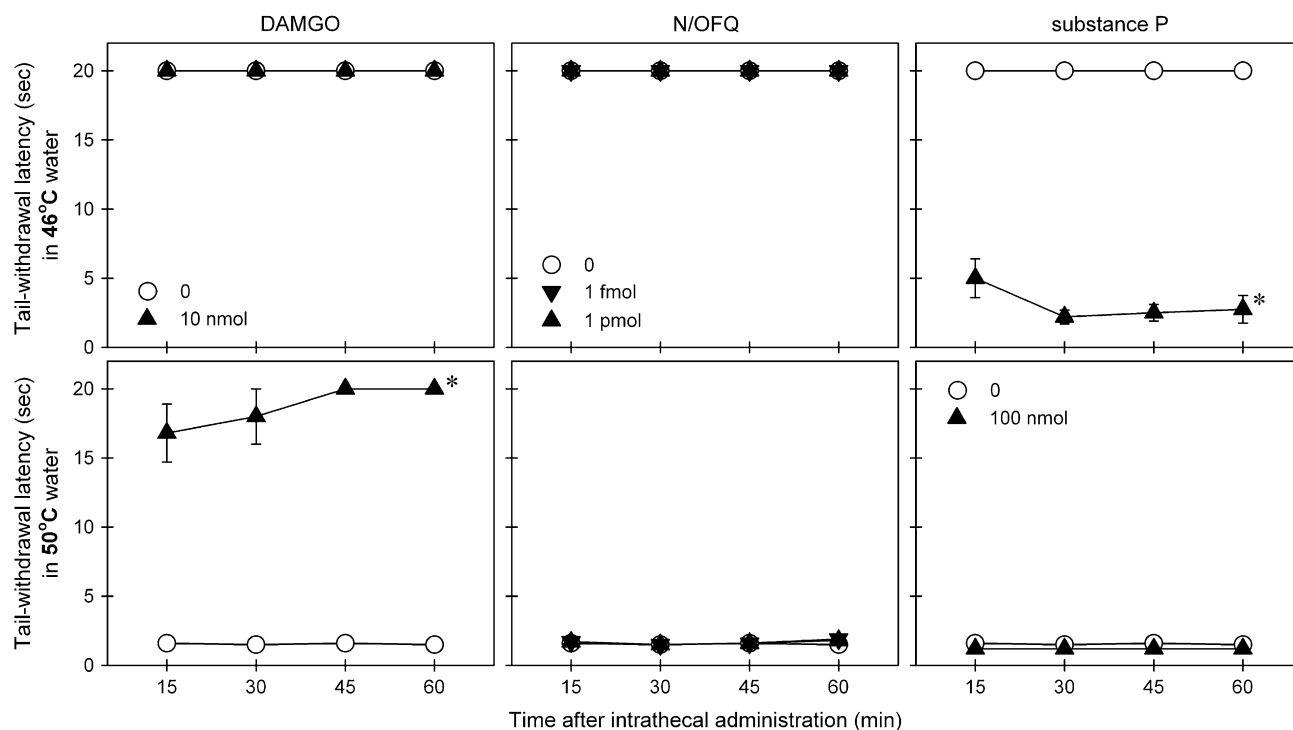
Scratching behavior, inferred to be a response to itch sensation,<sup>18,21</sup> was recorded on videotape while the monkeys were in their home cages. Each recording session was conducted for 15 minutes per test session. A scratch was defined as 1 short-duration (<1 second) episode of scraping contact of the forepaw or hind paw on the skin surface of other body parts. Scratches occurred repetitively at the same location. Scratching responses were scored by trained individuals who were blinded to experimental conditions. In addition, monkeys were rated for sedation and muscle relaxation according to 2 behavioral rating scales<sup>6</sup> while in their home cages. The monitoring of potential side effects was conducted by an observer at the last minute of each test session.

### Experimental Designs

The first part of the study was to determine behavioral responses of intrathecally administered N/OFQ over a wide range of ultra-low doses (ie, from 1 fmol to 1 nmol). In addition, effects of DAMGO and substance P were used as control conditions to compare with those of intrathecal N/OFQ. The doses of intrathecal DAMGO and substance P were selected based on a previous monkey study and our pilot study.<sup>21</sup> The tail-withdrawal latency in the temperatures 46°C (non-noxious) and 50°C (noxious) of warm water was used to detect potential hyperalgesic/pronociceptive and antinociceptive effects, respectively, in monkeys.<sup>19,22</sup> The second part of the study was to determine the degree of antinociception produced by intrathecal N/OFQ. The temperature 54°C of warm water represents a higher intensity of the nociceptive stimulus. The tail-withdrawal latency in both 50 and 54°C of warm water were used to characterize the antinociceptive effectiveness of intrathecal N/OFQ with increasing doses from 10 nmol to 1  $\mu$ mol. The third part of the study was to investigate how behaviorally active doses of N/OFQ modulated intrathecal morphine-induced antinociception and scratching responses. The dose of intrathecal morphine 50 nmol was selected based on previous studies,<sup>20,26</sup> showing that it produced maximal scratching responses and antinociception, and it could be used to detect whether intrathecal N/OFQ could interfere with morphine-mediated actions.

### Statistical Analysis

Mean values (mean  $\pm$  SEM) were calculated from individual values for all behavioral end points. Comparisons were made for the same monkeys across all test sessions in the same experiment. Data were analyzed by a 2-way



**Figure 1.** Comparison of warm water tail-withdrawal responses of intrathecally administered N/OFQ, DAMGO, and substance P. Top panels: Tail-withdrawal latency in 46°C water. Bottom panels: Tail-withdrawal latency in 50°C water. Behavioral responses were measured at 15-, 30-, 45-, and 60-minute time points after intrathecal administration of test compound, using a single dosing procedure. Each value represents mean  $\pm$  SEM ( $n = 6$ ). Symbols represent different dosing conditions for the same monkeys. Asterisk represents a significant difference from the vehicle condition for all time points ( $*P < .05$ ).

analysis of variance (ANOVA) followed by the Newman-Keuls test for multiple (post hoc) comparisons. For comparison of data at a single time point, data were analyzed by 1-way ANOVA followed by the Dunnett test for multiple comparisons. The criterion for significance was set at  $P < .05$ .

## Drugs

N/OFQ, morphine sulfate (National Institute on Drug Abuse, Bethesda, MD), DAMGO, and substance P (Sigma-Aldrich, St. Louis, MO) were dissolved in sterile water. Doses are presented in the compound forms listed above. For intrathecal administration, N/OFQ, morphine, or the mixture of N/OFQ and morphine was administered at a total volume of 1 mL. The detailed description for intrathecal drug delivery can be referred to previous studies.<sup>20,21</sup> All experiments using intrathecal administration were conducted with a 10-day inter-injection interval.

## Results

**Fig 1** illustrates distinct responses to nociceptive stimuli of monkeys receiving intrathecal administration of N/OFQ, DAMGO, and substance P. Intrathecal N/OFQ over a wide range of ultra-low doses (ie, from 1 fmol to 1 nmol) did not produce either hyperalgesic or antinociceptive responses (**Table 1**). In contrast, intrathecal substance P 100 nmol produced hyperalgesic responses in 46°C water [ $F(1,5) = 1025.2$ ;  $P < .05$ ] and intrathecal

DAMGO 10 nmol produced antinociceptive responses in 50°C water [ $F(1,5) = 335.9$ ;  $P < .05$ ].

**Fig 2** compares distinct behavioral responses of monkeys after intrathecal administration of N/OFQ, DAMGO, and substance P. Intrathecal N/OFQ over a wide dose range of ultra-low doses did not elicit scratching responses (**Table 1**). Although intrathecal substance P 100 nmol significantly produced hyperalgesic effects, this dose of substance P did not elicit scratching responses. In contrast, intrathecal DAMGO 10 nmol significantly evoked scratching responses [ $F(1,5) = 124.3$ ;  $P < .05$ ] in addition to its antinociceptive effects. Scratching evoked by intrathecal DAMGO peaked at the first observation period (ie, 15 minutes after intrathecal administration) and continued throughout the 1 hour observation period (**Fig 2** and **Table 1**). It is worth noting that intrathecal administration of N/OFQ, DAMGO, and substance P at these doses did not cause any observable side effects including sedation and muscle relaxation.

**Fig 3** shows behavioral responses of intrathecal N/OFQ at doses between 10 and 100 nmol. Intrathecal N/OFQ dose-dependently produced antinociceptive effects against a nociceptive stimulus, 50°C water [ $F(3,15) = 28.1$ ;  $P < .05$ ]. However, N/OFQ at these doses did not produce significant antinociception against a higher intensity of nociceptive stimulus, 54°C water and it did not elicit scratching responses under these conditions. For comparison, **Fig 4** shows behavioral responses of intrathecal N/OFQ at higher doses from 0.1 to 1  $\mu$ mol.

**Table 1. Behavioral Responses of Intrathecal Administration of N/OFQ Over a Wide Range of Ultra-Low Doses as Compared to a Single Dose of DAMGO and Substance P.**

| Compound/Dose | WARM WATER<br>TAIL-WITHDRAWAL LATENCY<br>(sec)* |             | ITCH/SCRATCHING†<br>NUMBER/15 MIN |
|---------------|---|-------------|-----------------------------------|
|               | 46°C  | 50°C        |                                   |
| N/OFQ         |   |             |                                   |
| 0 (vehicle)   | 20 ± 0‡   | 1.6 ± 0.1   | 50.0 ± 12.9                       |
| 1 fmol        | 20 ± 0  | 1.7 ± 0.1   | 33.8 ± 9.9                        |
| 10 fmol       | 20 ± 0  | 1.6 ± 0.2   | 49.3 ± 15.7                       |
| 100 fmol      | 20 ± 0  | 1.4 ± 0.2   | 44.3 ± 14.0                       |
| 1 pmol        | 20 ± 0  | 1.7 ± 0.1   | 57.2 ± 15.3                       |
| 10 pmol       | 20 ± 0  | 1.8 ± 0.1   | 57.5 ± 10.2                       |
| 100 pmol      | 20 ± 0  | 1.9 ± 0.2   | 41.0 ± 15.1                       |
| 1 nmol        | 20 ± 0  | 1.6 ± 0.2   | 35.2 ± 7.1                        |
| Substance P   |   |             |                                   |
| 100 nmol      | 4.9 ± 1.4§                                      | 1.2 ± 0.1   | 48.5 ± 8.6                        |
| DAMGO         |   |             |                                   |
| 10 nmol       | 20 ± 0  | 16.8 ± 2.1§ | 910.5 ± 103.9§                    |

\*The latency was measured at 15 min after intrathecal administration of test compound.

†The scratching number was scored between 15<sup>th</sup> and 30<sup>th</sup> min after intrathecal administration of test compound.

‡Each value represents mean ± S.E.M. (n = 6).

§The asterisk represents a significant difference from the vehicle condition ( $P < 0.05$ ).

All 3 doses of intrathecal N/OFQ produced significant antinociception against 50°C water [ $F(3,15) = 198.4$ ;  $P < .05$ ]. In addition, N/OFQ dose-dependently produced antinociceptive effects against 54°C water [ $F(3,15) = 15.1$ ,  $P < .05$ ] without evoking scratching responses. It is worth noting that intrathecal administration of N/OFQ at these doses did not cause any observable side effects including sedation and motor impairment.

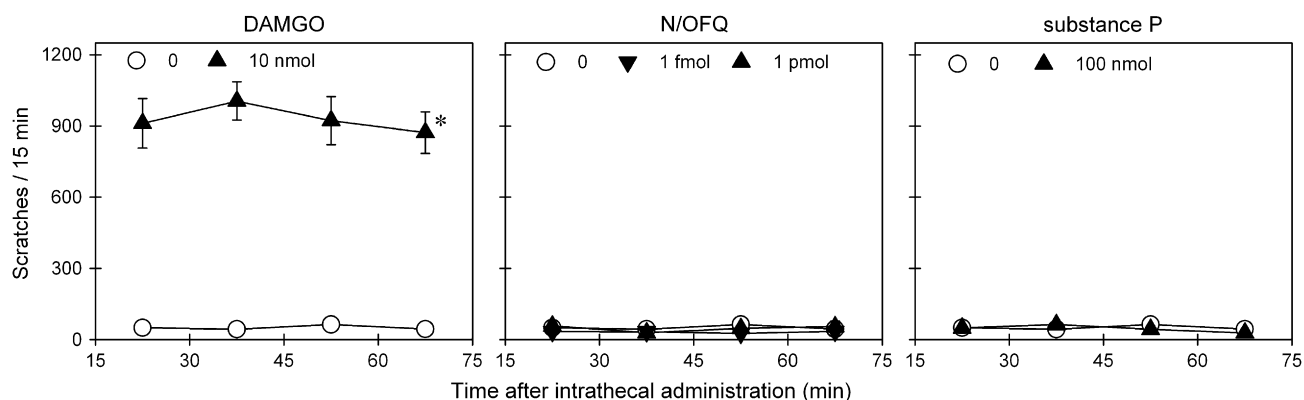
Fig 5 illustrates behavioral responses of intrathecal N/OFQ in combination with morphine. A single dose of intrathecal morphine 50 nmol produced antinociceptive effects against 50°C, not 54°C water (top 2 panels). This antinociceptive effect of intrathecal morphine was

accompanied by profound scratching responses (bottom panel). When N/OFQ was combined with intrathecal morphine, N/OFQ dose-dependently increased the mixture's antinociceptive effects against 54°C water [ $F(3,15) = 14.2$ ;  $P < .05$ ]. Under these conditions, increasing doses of N/OFQ did not attenuate intrathecal morphine-induced scratching responses.

## Discussion

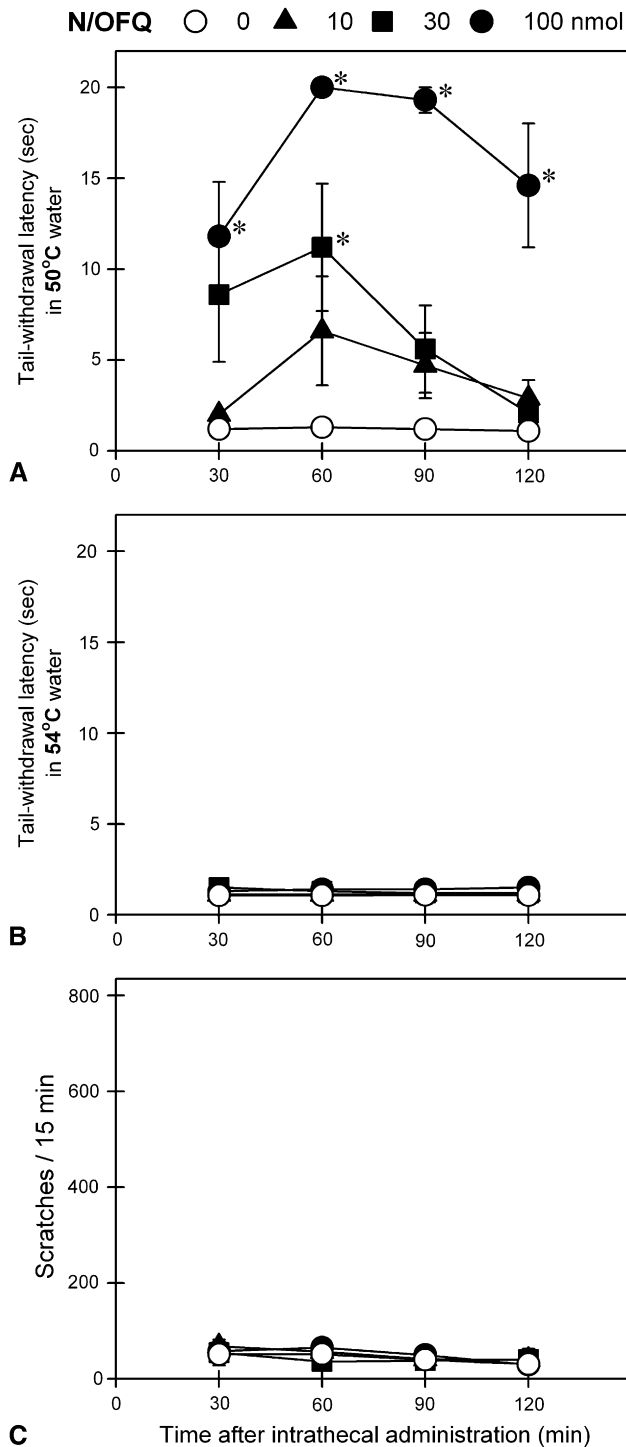
The present study showed that intrathecal administration of N/OFQ over a wide dose range (ie, from 1 fmol to 1  $\mu$ mol) produced thermal antinociception in the absence of hyperalgesia, scratching, sedation, and muscle relaxation. There were no sequelae to intrathecal N/OFQ, administered over several occasions consecutively in the same primates. For comparison, intrathecal administration of substance P 100 nmol significantly produced pronociceptive/hyperalgesic effects, manifested as reduced tail-withdrawal latencies in 46°C water. These results agree with rodent studies, indicating that intrathecal substance P causes hyperalgesic effects.<sup>27,30</sup> Intrathecal administration of substance P and N/OFQ both produced a similar degree of hyperalgesic effects, as shown by decreased response latency approximately for 2 to 3 seconds in rodents.<sup>30,39</sup> It has been suggested that intrathecal N/OFQ-induced hyperalgesia may be mediated by tachykinin NK1 receptors in the mouse spinal cord.<sup>39,40</sup> Although intrathecal N/OFQ did not produce hyperalgesic effects like intrathecal substance P in monkeys, more studies are warranted to elucidate the relationship of intrathecal substance P with other neurotransmitter systems in the modulation of nociceptive processing of the primate spinal cord.

In contrast, intrathecal administration of DAMGO 10 nmol significantly produced antinociceptive effects, manifested as elevated tail-withdrawal latencies in 50°C water. These effects are consistent with rodent studies, indicating that intrathecal DAMGO is a potent  $\mu$ -opioid antinociceptive agent.<sup>29,41</sup> By testing intrathecal N/OFQ, substance P, and DAMGO in the same animals, they displayed distinct effects on modulating the nociceptive threshold. Such findings may suggest that intrathecal N/



**Figure 2.** Comparison of itch/scratching responses of intrathecally administered N/OFQ, DAMGO, and substance P. Behavioral responses were scored for each 15-minute session after intrathecal administration of test compound, using a single dosing procedure. Each value represents mean ± SEM (n = 6). Symbols represent different dosing conditions for the same monkeys. Asterisk represents a significant difference from the vehicle condition ( $*P < .05$ ).





**Figure 3.** Behavioral responses of intrathecally administered N/OFQ at doses between 10 and 100 nmol. **A** and **B**, tail-withdrawal latency in 50° and 54°C water, respectively. **C**, itch/scratching responses for each 15-minute session crossing the time points, 30, 60, 90, or 120 minutes after intrathecal N/OFQ (ie, scratching number between 23rd and 38th minutes for the time point, 30 minutes). Each value represents mean  $\pm$  SEM ( $n = 6$ ). Symbols represent different experimental conditions for the same monkeys. Asterisk represents a significant difference from the vehicle condition at corresponding time point ( $*P < .05$ ).

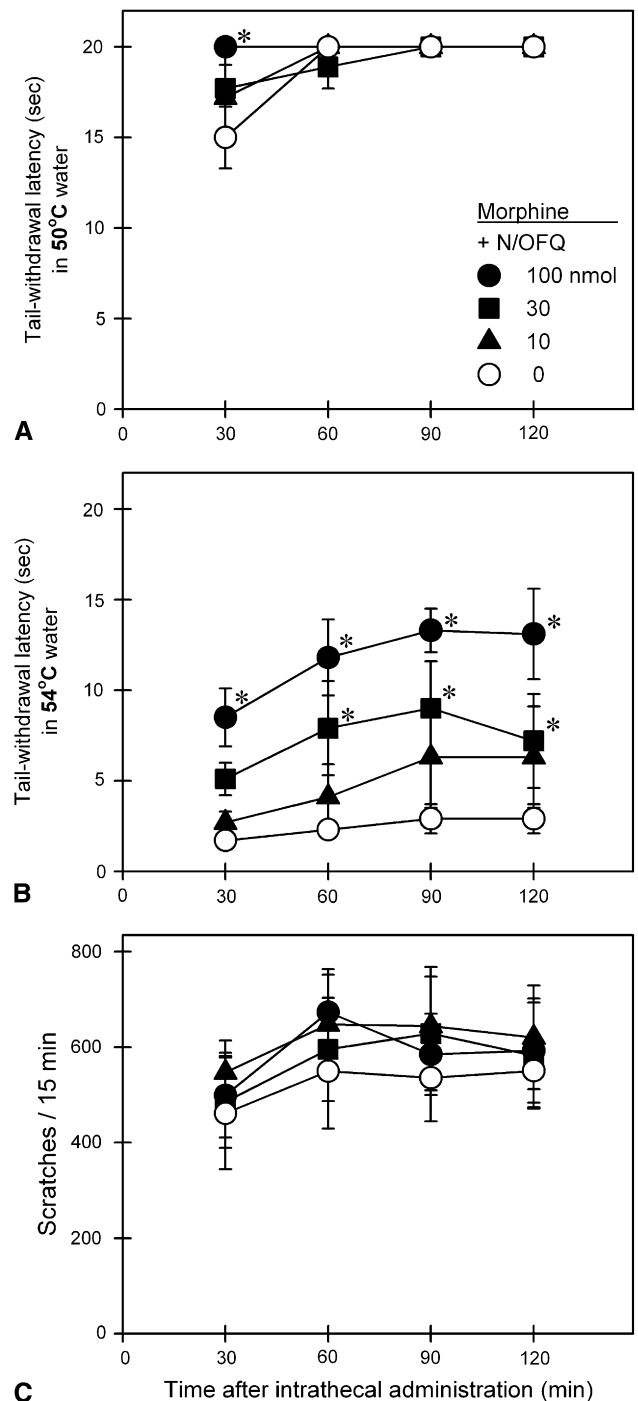
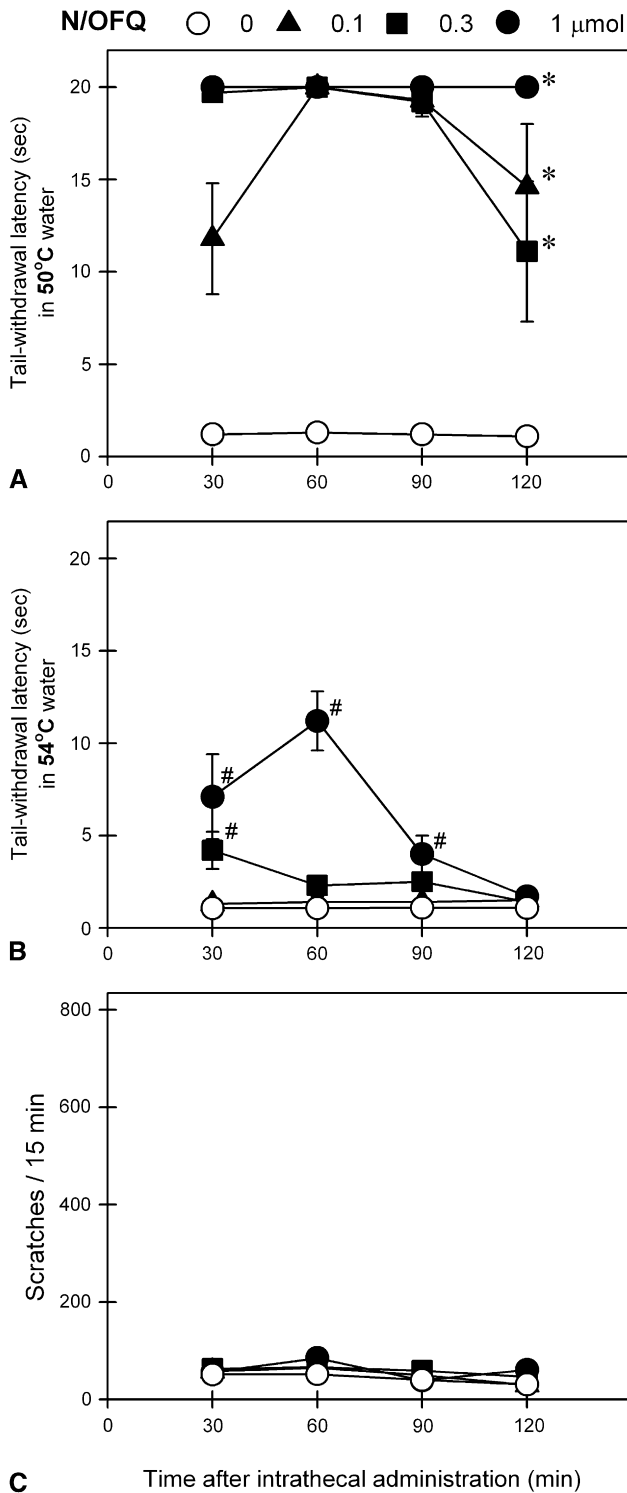
OFQ over a wide dose range does not produce pronociceptive/hyperalgesic responses in monkeys under this context.

Intrathecal administration of either N/OFQ or substance P did not significantly elicit scratching responses, but only intrathecal DAMGO elicited profound scratching responses (Fig 2 and Table 1). Behavioral responses of intrathecal DAMGO are expected because previous studies have demonstrated that antinociceptive doses of  $\mu$ -opioid receptor agonists elicited scratching responses in monkeys.<sup>18,20,26</sup> It is well known that intrathecal morphine produces pain relief accompanied by simultaneous itch sensation in humans.<sup>1,34</sup> These findings strongly support the notion that increased scratching responses in monkeys may represent a behavioral end point selective for itch sensation<sup>18,21</sup> and may suggest that intrathecal N/OFQ and substance P do not elicit itch sensation in primates.

It is interesting to know that intrathecal administration of substance P and N/OFQ both elicited scratching responses in rodents.<sup>3,13,15,40</sup> Nevertheless, rodents' scratching behavior may be neither necessary nor sufficient to be indicative of pain or itch sensation. For example, early studies showed that intrathecal substance P-induced scratching was not attenuated by pretreatment with analgesics, indicating that scratching is not pain-related.<sup>3,13</sup> In contrast, increased scratching is considered as a sign of chronic pain in arthritic rats.<sup>11</sup> Perhaps a series of behavioral responses including scratching, biting, and licking<sup>15,40</sup> after intrathecal substance P or N/OFQ represents a general behavioral spectrum in rodents under the state of pain or/and agitation, especially when additional measurements such as decreased response latency to a noxious stimulus were provided.<sup>30,39</sup> On the other hand, increased scratching is also considered as a behavioral response to itch sensation in rodents receiving pruritogenic agents.<sup>16,23,25</sup> Whether scratching behavior is pain-related or itch-related depends on the context. Several factors such as administration routes and species differences may also contribute to different results or interpretations in the behavioral pharmacology of itch. Therefore, it is very important to conduct more psychophysical studies in humans and functional studies in animals<sup>9,17,42</sup> to further integrate and elucidate the physiological role of each neurotransmitter in the modulation of itch and pain sensation.

Intrathecal administration of N/OFQ at the dose range from 10 nmol to 1  $\mu$ mol dose-dependently produced antinociception against a noxious stimulus in 2 intensities (Figs 3 and 4). The magnitude of N/OFQ's antinociceptive effects in this assay is potentially similar to that of clinically available  $\mu$ -opioid analgesics, such as nalbuphine, morphine, and fentanyl.<sup>5,18,43</sup> Importantly, these antinociceptive doses of intrathecal N/OFQ did not elicit scratching responses. As previously demonstrated, intrathecal N/OFQ-induced antinociception was blocked by pretreatment with a selective NOP receptor antagonist, J-113397<sup>33</sup> but not by a classic opioid receptor antagonist, naltrexone.<sup>22</sup> These findings together suggest that intrathecal N/OFQ or other NOP receptor agonists may have the therapeutic potential as spinal analgesics without side effects derived from  $\mu$ -opioid receptor agonists. The degree of antinociception produced by an





**Figure 5.** Behavioral responses of intrathecally administered N/OFQ in combination with morphine. Open circles represent the effects of intrathecal morphine 50 nmol alone. Other symbols represent effects of the same dose of morphine in combination with different doses of N/OFQ in the same monkeys. Each value represents mean  $\pm$  SEM ( $n = 6$ ). Asterisk represents a significant difference from the control condition (ie, intrathecal morphine alone) at corresponding time point ( $*P < .05$ ). See Fig 3 for other details.

experimental compound depends on its intrinsic efficacy and the nociceptive stimulus intensity.<sup>12,35,38</sup> Future studies are needed to further investigate whether intrathecal N/OFQ or other NOP receptor agonists produce the same degree of antinociception as  $\mu$ -opioid receptor agonists in

monkeys under different pain modalities. In particular, long-lasting NOP receptor agonists<sup>32,37</sup> such as UFP-112 have been identified, and it would be important to study such agonists in the context of spinal delivery in primates.

When N/OFQ was combined with a single dose of intrathecal morphine, this addition potentiated intrathecal morphine-induced antinociception, manifested as elevated tail-withdrawal latencies in 54°C water, by increasing the dose of N/OFQ (Fig 5). Interestingly, addition of intrathecal N/OFQ did not attenuate intrathecal morphine-elicited scratching responses. These results may indicate that intrathecal N/OFQ potentiates morphine-induced antinociception without producing motor-related side effects because monkeys still display profound scratching responses. Furthermore, in contrast to anti-morphine actions of supraspinal N/OFQ,<sup>7,45</sup> intrathecal N/OFQ did not produce anti-morphine actions, indicating that N/OFQ has different actions on spinal versus supraspinal sites.<sup>45</sup> It would be reasonable to expect that intrathecal administration of a mixture of morphine with NOP receptor agonists produces antinociceptive effectiveness with fewer side effects. It also would be interesting to investigate the development of tolerance

to antinociceptive effects of spinally administered morphine or/and NOP receptor agonists in future studies.

In summary, this study reveals a unique functional profile of intrathecal N/OFQ in primates. Unlike dual actions (ie, both pronociceptive and antinociceptive effects) of intrathecal N/OFQ observed in rodents, intrathecal N/OFQ over a wide dose range only produced antinociception. More importantly, intrathecal N/OFQ did not produce anti-morphine actions when it was combined with intrathecal morphine. The therapeutic potential of N/OFQ and the NOP receptors has been emphasized for its broad medical applications.<sup>24,45</sup> Given that intrathecal N/OFQ produces antinociception without eliciting itch/scratching responses in monkeys, N/OFQ or other NOP receptor agonists represent a viable target as spinal analgesics for future clinical trials.

## Acknowledgments

We thank John Pascoe, Kathryn Craig, Katie Hamelink, and Karthik Murali for technical assistance. All authors declare that they have no conflicts of interest.

## References

- Bailey PL, Rhondeau S, Schafer PG, Lu JK, Timmins BS, Foster W, Pace NL, Stanley TH: Dose-response pharmacology of intrathecal morphine in human volunteers. *Anesthesiology* 79:49-59, 1993
- Berthele A, Platzer S, Dworzak D, Schadrack J, Mahal B, Butter A, Aßmus HP, Wurster K, Zieglansberger W, Conrad B, Tolle TR: [3H]-Nociceptin ligand-binding and nociception opioid receptor mRNA expression in the human brain. *Neuroscience* 121:629-640, 2003
- Bossut D, Frenk H, Mayer DJ: Is substance P a primary afferent neurotransmitter for nociceptive input? II: Spinalization does not reduce and intrathecal morphine potentiates behavioral responses to substance P. *Brain Res* 455:232-239, 1988
- Bridge KE, Wainwright A, Reilly K, Oliver KR: Autoradiographic localization of [125I]Tyr14]nociceptin/orphanin FQ binding sites in macaque primate CNS. *Neuroscience* 118:513-523, 2003
- Butelman ER, Lewis JW, Woods JH: Methoclocinnamox: Agonist and antagonist effects of a novel long-lasting opioid in rhesus monkeys. *J Pharmacol Exp Ther* 279:934-938, 1996
- Butelman ER, Harris TJ, Kreek MJ: Effects of E-2078, a stable dynorphin A(1-8) analog, on sedation and serum prolactin levels in rhesus monkeys. *Psychopharmacology* 147:73-80, 1999
- Calò G, Rizzi A, Marzola G, Guerrini R, Salvadori S, Beani L, Regoli D, Bianchi C: Pharmacological characterization of the nociceptin receptor mediating hyperalgesia in the mouse tail withdrawal assay. *Br J Pharmacol* 125:373-378, 1998
- Cousins MJ, Mather LE: Intrathecal and epidural administration of opioids. *Anesthesiology* 61:276-310, 1984
- Davidson S, Zhang X, Yoon CH, Khasabov SG, Simone DA, Giesler G Jr: The itch-producing agents histamine and cowhage activate separate populations of primate spinothalamic tract neurons. *J Neurosci* 27:10007-10014, 2007
- DeBalli P, Breen TW: Intrathecal opioids for combined spinal-epidural analgesia during labour. *CNS Drugs* 17:889-904, 2003
- De Castro-Costa M, Gybels J, Kupers R, Van Hees J: Scratching behavior in arthritic rats: A sign of chronic pain or itch? *Pain* 29:123-131, 1987
- Dirig DM, Yaksh TL: Differential right shifts in the dose-response curve for intrathecal morphine and sufentanil as a function of stimulus intensity. *Pain* 62:321-328, 1995
- Frenk H, Bossut D, Urca G, Mayer DJ: Is substance P a primary afferent neurotransmitter for nociceptive input? I: Analysis of pain-related behaviors resulting from intrathecal administration of substance P and 6 excitatory compounds. *Brain Res* 455:223-231, 1988
- Ganesh A, Maxwell LG: Pathophysiology and management of opioid-induced pruritus. *Drugs* 67:2323-2333, 2007
- Inoue M, Shimohira I, Yoshida A, Zimmer A, Takeshima H, Sakurada T, Ueda H: Dose-related opposite modulation by nociceptin/orphanin FQ of substance P nociception in the nociceptors and spinal cord. *J Pharmacol Exp Ther* 291:308-313, 1999
- Jinks SL, Carstens E: Responses of superficial dorsal horn neurons to intradermal serotonin and other irritants: Comparison with scratching behavior. *J Neurophysiol* 87:1280-1289, 2002
- Johanek LM, Meyer RA, Hartke T, Hobelmann JG, Maine DN, LaMotte RH, Ringkamp M: Psychophysical and physiological evidence parallel afferent pathways mediating the sensation of itch. *J Neurosci* 27:7490-7497, 2007
- Ko MCH, Naughton NN: An experimental itch model in monkeys: Characterization of intrathecal morphine-induced scratching and antinociception. *Anesthesiology* 92:795-805, 2000

19. Ko MCH, Naughton NN, Traynor JR, Song MS, Woods JH, Rice KC, McKnight AT: Orphanin FQ inhibits capsaicin-induced thermal nociception in monkeys by activation of peripheral ORL1 receptors. *Br J Pharmacol* 135:943-950, 2002
20. Ko MCH, Lee H, Song MS, Sobczyk-Kojiro K, Mosberg HI, Kishioka S, Woods JH, Naughton NN: Activation of  $\kappa$ -opioid receptors inhibits pruritus evoked by subcutaneous or intrathecal administration of morphine in monkeys. *J Pharmacol Exp Ther* 305:173-179, 2003
21. Ko MCH, Song MS, Edwards T, Lee H, Naughton NN: The role of central mu opioid receptors in opioid-induced itch in primates. *J Pharmacol Exp Ther* 310:169-176, 2004
22. Ko MCH, Wei H, Woods JH, Kennedy RT: Effects of intrathecally administered nociceptin/orphanin FQ in monkeys: Behavioral and mass spectrometric studies. *J Pharmacol Exp Ther* 318:1257-1264, 2006
23. Kuraishi Y, Nagasawa T, Hayashi K, Satoh M: Scratching behavior induced by pruritogenic but not algisogenic agents in mice. *Eur J Pharmacol* 275:229-233, 1995
24. Lambert DG: The nociceptin/orphanin FQ receptor: A target with broad therapeutic potential. *Nat Rev Drug Discov* 7:694-710, 2008
25. Lee H, Naughton NN, Woods JH, Ko MCH: Characterization of scratching responses in rats following centrally administered morphine or bombesin. *Behav Pharmacol* 14: 501-508, 2003
26. Lee H, Naughton NN, Woods JH, Ko MC: Effects of butorphanol on morphine-induced itch and analgesia in primates. *Anesthesiology* 107:478-485, 2007
27. Matsumura H, Sakurada T, Hara A, Sakurada S, Kisara K: Characterization of the hyperalgesic effect induced by intrathecal injection of substance P. *Neuropharmacology* 24: 421-426, 1985
28. Meunier J-C, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P, Butour J-L, Guillemot J-C, Ferrara P, Monsarrat B, Mazarguil H, Vassart G, Parmentier M, Costentin J: Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. *Nature* 377: 532-535, 1995
29. Mjanger E, Yaksh TL: Characteristics of dose-dependent antagonism by  $\beta$ -funaltrexamine of the antinociceptive effects of intrathecal mu agonists. *J Pharmacol Exp Ther* 258:544-550, 1991
30. Moochhala SM, Sawynok J: Hyperalgesia produced by intrathecal substance P and related peptides: Desensitization and cross desensitization. *Br J Pharmacol* 82:381-388, 1984
31. Neal CR Jr., Akil H, Watson SJ: Expression of orphanin FQ and the opioid receptor-like (ORL1) receptor in the developing human and rat brain. *J Chem Neuroanat* 22:219-249, 2001
32. Okada K, Sujaku T, Chuman Y, Nakashima R, Nose T, Costa T, Yamada Y, Yokoyama M, Nagahisa A, Shimohigashi Y: Highly potent nociception analog containing the Arg-Lys triple repeat. *Biochem Biophys Res Commun* 278:493-498
33. Ozaki S, Kawamoto H, Itoh Y, Miyaji M, Azuma T, Ichikawa D, Nambu H, Iguchi T, Iwasawa Y, Ohta H: In vitro and in vivo pharmacological characterization of J-113397, a potent and selective non-peptidyl ORL1 receptor antagonist. *Eur J Pharmacol* 402:45-53, 2000
34. Palmer CM, Emerson S, Volgoropolous D, Alves D: Dose-response relationship of intrathecal morphine for postcesarean analgesia. *Anesthesiology* 90:437-444, 1999
35. Parsons CG, Headley PM: Spinal antinociceptive actions of mu- and kappa-opioids: The importance of stimulus intensity in determining "selectivity" between reflexes to different modalities of noxious stimulus. *Br J Pharmacol* 98: 523-532, 1998
36. Reinscheid RK, Nothacker H-P, Bourson A, Ardati A, Henningsen RA, Bunzow JR, Grandy DK, Langen H, Monsma FJ Jr., Civelli O, Orphanin FQ: A neuropeptide that activates an opioidlike G protein-coupled receptor. *Science* 270:792-794, 1995
37. Rizzi A, Spagnolo B, Wainford RD, Fischette C, Guerrini R, Marzola G, Baldisserotto A, Salvadori S, Regoli D, Kapusta DR, Calo G: In vitro and in vivo studies on UFP-112, a novel potent and long lasting agonist selective for the nociception/orphanin FQ receptor. *Peptides* 28: 1240-1251, 2007
38. Saeki S, Yaksh TL: Suppression of nociceptive responses by spinal mu opioid agonists: Effects of stimulus intensity and agonist efficacy. *Anesth Analg* 77:265-274, 1993
39. Sakurada C, Sakurada S, Katsuyama S, Sasaki J, Tan-No K, Sakurada T: Involvement of tachykinin NK1 receptors in nociceptin-induced hyperalgesia in mice. *Brain Res* 841: 85-92, 1999
40. Sakurada T, Katsuyama S, Sakurada S, Inoue M, Tan-No K, Kisara K, Sakurada C, Ueda H, Sasaki J: Nociception-induced scratching, biting, and licking in mice: Involvement of spinal NK1 receptors. *Br J Pharmacol* 127:1712-1718, 1999
41. Xie H, Woods JH, Traynor JR, Ko MC: The spinal antinociceptive effects of endomorphins in rats: Behavioral and G protein functional studies. *Anesth Analg* 106:1873-1881, 2008
42. Yosipovitch G, Carstens E: Report from the 4th international workshop for the study of itch. *J Invest Dermatol* 128:256-257, 2008
43. Walker EA, Butelman ER, DeCosta BR, Woods JH: Opioid thermal antinociception in rhesus monkeys: Receptor mechanisms and temperature dependency. *J Pharmacol Exp Ther* 267:280-286, 1993
44. Witta J, Palkovits M, Rosenberger J, Cox BM: Distribution of nociception/orphanin FQ in adult human brain. *Brain Res* 997:24-29, 2004
45. Zeilhofer HU, Calo G: Nociceptin/orphanin FQ and its receptor-potential targets for pain therapy? *J Pharmacol Exp Ther* 306:423-429, 2003

# Behavioral Effects of a Synthetic Agonist Selective for Nociceptin/Orphanin FQ Peptide Receptors in Monkeys

Mei-Chuan Ko<sup>\*1,2</sup>, James H Woods<sup>1</sup>, William E Fantegrossi<sup>3</sup>, Chad M Galuska<sup>4</sup>, Jürgen Wichmann<sup>5</sup> and Eric P Prinsen<sup>5</sup>

<sup>1</sup>Department of Pharmacology, University of Michigan, Ann Arbor, MI, USA; <sup>2</sup>Department of Psychology and Institute of Neuroscience, National Cheng Chi University, Taipei, Taiwan; <sup>3</sup>Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR, USA; <sup>4</sup>Department of Psychology, College of Charleston, Charleston, SC, USA; <sup>5</sup>F.Hoffmann-La Roche Ltd, Basel, Switzerland

Behavioral effects of a nonpeptidic NOP (nociceptin/orphanin FQ Peptide) receptor agonist, Ro 64-6198, have not been studied in primate species. The aim of the study was to verify the receptor mechanism underlying the behavioral effects of Ro 64-6198 and to systematically compare behavioral effects of Ro 64-6198 with those of a  $\mu$ -opioid receptor agonist, alfentanil, in monkeys. Both Ro 64-6198 (0.001–0.06 mg/kg, s.c.) and alfentanil (0.001–0.06 mg/kg, s.c.) produced antinociception against an acute noxious stimulus (50°C water) and capsaicin-induced allodynia. An NOP receptor antagonist, J-113397 (0.01–0.1 mg/kg, s.c.), dose-dependently produced rightward shifts of the dose–response curve of Ro 64-6198-induced antinociception. The apparent  $pA_2$  value of J-113397 was 8.0. Antagonist studies using J-113397 and naltrexone revealed that Ro 64-6198 produced NOP receptor-mediated antinociception independent of  $\mu$ -opioid receptors. In addition, alfentanil dose-dependently produced respiratory depression and itch/scratching responses, but antinociceptive doses of Ro 64-6198 did not produce such effects. More important, Ro 64-6198 did not produce reinforcing effects comparable with those of alfentanil, cocaine, or methohexital under self-administration procedures in monkeys. These results provide the first functional evidence that the activation of NOP receptors produces antinociception without reinforcing effects in primates. Non-peptidic NOP receptor agonists may have therapeutic value as novel analgesics without abuse liability in humans. *Neuropsychopharmacology* advance online publication, 11 March 2009; doi:10.1038/npp.2009.33

**Keywords:** opioid; antinociception; self-administration; analgesic; abuse liability

## INTRODUCTION

Opioid analgesics are the most effective and widely used drugs for pain management; the most clinically used opioids are  $\mu$ -opioid receptor agonists (Zollner and Stein, 2007). However, there are several side effects associated with the use of  $\mu$ -opioid agonists. These include constipation, respiratory depression, and itch/pruritus (Zollner and Stein, 2007). Importantly, the abuse liability derived from  $\mu$ -opioid agonists has been and remains a serious public health concern and limits the opioid analgesics' value for pain management (Cicero *et al*, 2007; Katz *et al*, 2007). Research to identify potential analgesics with fewer side effects and reduced abuse liability is pivotal to advances in health care of all individuals.

Given that the neuroanatomical and physiological aspects of opioid receptors are similar between humans and monkeys

(Kuhar *et al*, 1973; Mansour *et al*, 1988; Peckys and Landwehrmeyer, 1999), the functions of opioid receptor subtypes can be investigated in nonhuman primates using a variety of behavioral assays and experimental compounds that are likely to be relevant to humans. In particular, the self-administration assay in monkeys has been used extensively, and it provides useful information for the abuse liability of drugs in humans (Weerts *et al*, 2007). Depending on the experimental schedules, most abused drugs in humans have been shown to have reinforcing effects in monkey self-administration procedures (Winger *et al*, 1975; Ator and Griffiths, 1987; Weerts *et al*, 2007). Although neither  $\kappa$ - nor  $\delta$ -opioid agonists produce reinforcing effects, drugs in these categories do not have promising pharmacological profiles as strong analgesics because of their undesirable side effects. Centrally penetrating  $\kappa$ -opioid agonists' antinociceptive effects are compromised by sedation, and  $\delta$ -opioid agonists are weak analgesics limited by potential convulsant effects (Dykstra *et al*, 1987; Negus *et al*, 1998).

The NOP receptor, previously called the ORL1 receptor, is defined as the fourth member within the opioid receptor family by the International Union of Pharmacology (Mollereau *et al*, 1994; Foord *et al*, 2005). An endogenous peptide selective for the NOP receptor, nociceptin/orphanin

\*Correspondence: Dr MC Ko, Department of Pharmacology, University of Michigan Medical School, 1301 MSRB III, Ann Arbor, MI 48109-5632, USA. Tel.: 1 734 647 3119, Fax: 1 734 764 7118; E-mail: mko@umich.edu

Received 1 December 2008; revised 8 February 2009; accepted 10 February 2009

FQ (N/OFQ), has been identified and shown to have similar actions as other opioid peptides at the cellular level (Meunier *et al*, 1995; Reinscheid *et al*, 1995). Although activation of supraspinal NOP receptors may produce hyperalgesic effects (Meunier *et al*, 1995; Rizzi *et al*, 2007), most studies have shown that activation of peripheral and spinal NOP receptors produces antinociceptive effects in a variety of pain models in rodents (Erb *et al*, 1997; Zeilhofer and Calo, 2003; Obara *et al*, 2005). Interestingly, both peripheral and spinal administration of N/OFQ produce antinociceptive effects in monkeys, indicating a potential therapeutic value of NOP receptor agonists as analgesics (Ko *et al*, 2002b, 2006).

The development of a selective nonpeptidic NOP receptor agonist, Ro 64-6198 (Jenck *et al*, 2000; Wichmann *et al*, 2000), and antagonist, J-113397 (Kawamoto *et al*, 1999), provides an opportunity to study integrated behavioral effects of a NOP receptor agonist in animals following systemic administration (Chiou *et al*, 2007; Shoblock, 2007). However, to date, there is no study investigating the behavioral pharmacological actions of Ro 64-6198 in primates. In particular, it is important to investigate whether Ro 64-6198 produces any reinforcing effect/abuse liability in monkey self-administration procedures. Therefore, the aim of the study was to clarify the receptor mechanism underlying Ro 64-6198-induced behavioral responses. Antinociceptive effects of Ro 64-6198 were further examined using different pain modalities and various behavioral assays were applied to systematically compare effects between Ro 64-6198 and alfentanil, a  $\mu$ -opioid receptor agonist, in monkeys.

## MATERIALS AND METHODS

### Subjects

Twenty seven adult gonadally intact male and female rhesus monkeys (*Macaca mulatta*) with body weights ranging between 6.6 and 11.7 kg were used. Twelve monkeys participated in the antinociception and itch/scratching studies, and another six monkeys participated in the respiration study. The remaining nine monkeys were used in the self-administration study. The monkeys were housed individually with free access to water and were fed approximately 25–30 biscuits (Purina Monkey Chow, product No. 5045; Ralston Purina, St Louis, MO) and fresh fruit daily. No monkey had exposure to any opioid receptor agonist or antagonist for 1 month before this study. The monkeys were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care. The studies were conducted in accordance with the University Committee on the Use and Care of Animals at the University of Michigan and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health (National Academy Press, Washington DC, revised 1996).

## EXPERIMENTAL PROCEDURES

### Antinociception

The warm water (50°C) tail-withdrawal assay was used to evaluate thermal antinociceptive effects of the test

compound (Ko *et al*, 1998a). Briefly, monkeys were seated in primate restraint chairs, and the lower part of their shaved tails (approximately 15 cm) were immersed in a thermal flask containing water maintained at either 42, 46, or 50°C. Tail-withdrawal latencies were measured using a computerized timer by an experimenter who was blinded to experimental conditions. In each test session, monkeys were tested once with three temperatures given in a random order, and only the 50°C water was tested twice to confirm the full antinociceptive effect. If the monkeys did not remove their tails within 20 s, the flask was removed and a maximum time of 20 s was recorded. Test sessions began with control determinations at each temperature. Then, the test compound was administered subcutaneously by a cumulative dosing procedure with a 30-min interinjection interval. Subsequent tail-withdrawal latencies were determined starting 20 min after each injection.

The NOP receptor antagonist potency of J-113397 against Ro 64-6198-induced antinociception was determined by giving subjects different doses of s.c. J-113397 (0.01, 0.03, and 0.1 mg/kg) for *in vivo* apparent  $pA_2$  analysis. In particular, the dose-response curve of s.c. Ro 64-6198 for antinociception was redetermined 15 min after pretreatment with a single dose of J-113397. A single dose of naltrexone (0.03 mg/kg) and J-113397 (0.1 mg/kg) was used to compare their antagonist effects against both alfentanil- and Ro 64-6198-induced antinociception. The dose and pretreatment time (ie, 15 min) for both naltrexone and J-113397 were chosen based on an earlier study (Ko *et al*, 1998a).

The tail-withdrawal latency in 46°C water following 0.1 mg of capsaicin administration was measured to evaluate the potential antiallodynic effects of analgesics (Ko *et al*, 1998b, 2002b). The procedure for studying thermal allodynia was slightly different from the general procedure for measuring thermal antinociception. The dose-response studies were measured by using a single-dosing procedure. The 46°C water was the thermal threshold for these subjects for expressing allodynic responses following the local injection of the capsaicin (Ko *et al*, 1998b, 2002b). After the chemical was administered s.c. in the tail, it dose-dependently produced thermal allodynia that peaked 15 min following the injection. This allodynic response was manifested as a reduced tail-withdrawal latency from a maximum value of 20 s to approximately 2–3 s in 46°C water. The test compounds, Ro 64-6198 and alfentanil, were administered s.c. 15 min before the capsaicin administration.

### Scratching Responses

Scratching responses, inferred as an itch sensation (Ko *et al*, 2004), were recorded on videotapes when monkeys were in their home cages. The test compound was administered i.m. by a cumulative dosing procedure with a 30-min interinjection interval. Each recording session was conducted for 15 min/test session (ie, from 15 to 30 min for each drug injection cycle). A scratch was defined as one short-duration (<1 s) episode of scraping contact of the forepaw or hindpaw on the skin surface of other body parts. Scratching responses were scored by trained individuals who were blinded to experimental conditions. In addition, sedation was monitored by cumulative time for eye closure or lying down at the bottom of the cage. Both scratching

and sedation end points were summed into one score per session.

### Respiratory Function

The apparatus is similar to that described previously (Butelman *et al*, 1993). The monkey was seated in a primate restraint chair, enclosed within a sound-attenuating chamber. A rectangular helmet ( $13.5 \times 17.0 \times 13.5$  cm) was placed over the head of the monkey and sealed around its neck by two closely fitting latex shields. Gas (either air or a mixture of 5% CO<sub>2</sub> in air) flowed into the helmet and was pumped out at a rate of 8 l/min. The monkeys' breathing produced changes in pressure inside the helmet that were measured with a pressure transducer connected to a polygraph (Grass Model 7). The data were recorded on a polygraph trace and in a microprocessor (IBM PC) through an analog-to-digital converter. The polygraph integrator was connected to a computer, which analyzes the data collected over a 3-min period. The rate of breathing ( $f$ , respiratory frequency) is determined directly. The minute volume ( $V_E$ ), the number of liters of air inspired per min, is determined from the integration of the plethysmograph system. The test compound was given i.m. in a cumulative dosing procedure, the test session contained 5–6 consecutive cycles of exposure to air. Each cycle was 30 min, which included a 23-min exposure to air alone and a 7-min exposure to 5% CO<sub>2</sub> mixed in air. The test compound was administered in the beginning of each test cycle and the doses were increased by a 0.25 or 0.5 log unit throughout the test sessions.

### Self-Administration

Three groups of monkeys ( $n = 3$  per group), with baselines of either alfentanil, cocaine, or methohexital self-administration were used to evaluate the reinforcing effects of Ro 64-6198. The common elements of the groups were that drug availability was signaled by a red stimulus light in the monkeys' home cages, and a fixed number of responses on a lever located beneath the stimulus light resulted in an infusion of drug or saline. The red light was extinguished and a green light was paired with the infusion. The red light remained off for a brief period after the infusion (timeout), during which time responding on the lever had no programmed consequence. Ro 64-6198 or saline was substituted for the baseline drug no more often than once every fourth session; two 2-h sessions were scheduled each day. In the two groups with alfentanil and cocaine baselines, each infusion followed 30 responses, which in turn, was followed by a 45-s timeout. In addition, each session comprised four components, each 25 min or 20 infusions in duration. The duration of the infusion pump, and therefore, the dose of the drug, was varied across components, so that dose-response observations could be made in each session (Winger *et al*, 1992).

A more rigorous evaluation of the reinforcing effects of Ro 64-6198 was made in the monkeys that had sodium methohexital as a baseline drug. In this case, a single dose of drug (0.1 mg/kg methohexital as baseline) was available throughout each twice-daily session on an FR 10–60 s schedule. The simpler schedule with a smaller response requirement as well as a comparison with a drug that is less

reinforcing than cocaine or alfentanil was used in these animals to increase the possibility of observing a reinforcing effect of Ro 64-6198.

### Data Analysis

Mean values (mean  $\pm$  SEM) were calculated from all behavioral endpoint. Comparisons were made for the same monkeys across all test sessions in the same experiment. For the dose-response curves for antinociception, individual tail-withdrawal latencies were converted to percentage of maximum possible effect. The formula of the percentage of maximum possible effect is defined as  $((\text{test latency} - \text{control latency}) / (\text{cutoff latency} - \text{control latency})) \times 100$ . ED<sub>50</sub> values were calculated by least-squares regression with the portion of the dose-response curves spanning the 50% maximum possible effect. The 95% confidence limits were also determined. Mean ED<sub>50</sub> values were considered to be significantly different when their 95% confidence limits did not overlap. For *in vivo* apparent pA<sub>2</sub> analysis (ie, multiple doses of antagonist), dose ratios between dose and response curves were analyzed in a Schild plot, and the mean J-113397 pA<sub>2</sub> value was averaged from the individual values following linear regression lines in the Schild plot. In addition, apparent pK<sub>B</sub> values were determined for a single dose of antagonist by using a modified equation,  $pK_B = -\log (B / (\text{dose ratio} - 1))$ , where  $B$  equals the antagonist dose in moles/kg. Mean pK<sub>B</sub> values  $\pm$  95% confidence limits were averaged from individual pK<sub>B</sub> values for J-113397 and naltrexone.

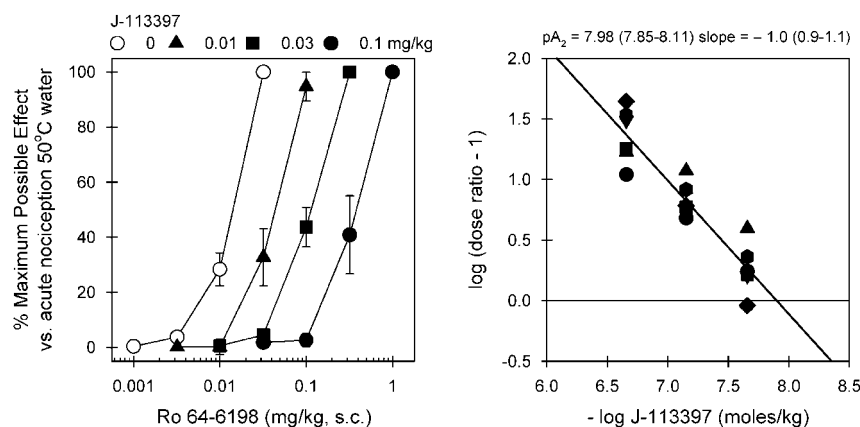
Mean number of injections earned or response rates for each dose of self-administered drug were calculated by averaging the results of each substitution trial for a given dose across all experimental subjects. The one-way ANOVA was conducted for data obtained from scratching, respiration, and self-administration experiments. Where appropriate, *post hoc* comparisons using the Tukey's test were made between the drug effect and the vehicle effect. The criterion for significance was set at  $P < 0.05$ .

### Drugs

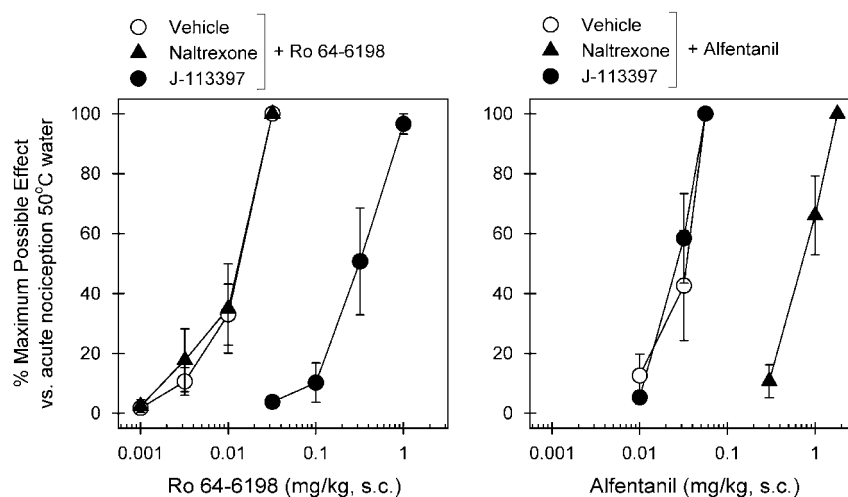
Alfentanil HCl, naltrexone HCl, (–)cocaine HCl, and (+)J-113397, provided by the National Institute on Drug Abuse (Bethesda, MD), were dissolved in sterile water. Ro 64-6198, provided by F. Hoffmann-La Roche AG (Basel, Switzerland), was dissolved in a solution of DMSO/Tween 80/sterile water in a ratio of 1:1:8. Capsaicin (Sigma, St Louis, MO) was dissolved in a solution of ethanol/Tween80/saline in a ratio of 1:1:8, and it was administered s.c. in the terminal 3–6 cm of the tail with constant 0.1 ml volume. Methohexital, purchased from Ace Surgical Supplies (Brockton, MA), was diluted with sterile water. Doses are presented in the compound forms listed above. For systemic administration in antinociception, scratching, and respiration experiments, all test compounds were administered at a volume of 0.1 ml/kg.

### RESULTS

Figure 1 illustrates the antagonist effect of J-113397 against Ro 64-6198-induced antinociception in 50°C water.



**Figure 1** *In vivo* antagonist potency of J-113397 against Ro 64-6198-induced antinociception in monkeys. Left panel, antagonist effects of s.c. J-113397 on the dose-response curve of Ro 64-6198-induced antinociception in 50°C water. Each data point represents a mean  $\pm$  SEM ( $n = 6$ ). Right panel, a Schild plot for J-113397. Abscissa, negative log unit for J-113397 in moles/kg. Ordinate, log of (dose ratio - 1). Each point was converted from individual dose ratio for each dosing condition presented in the left panel. Closed symbols represent different subjects. The mean  $pA_2$  value and slope of J-113397 are shown with 95% confidence limits in parentheses.



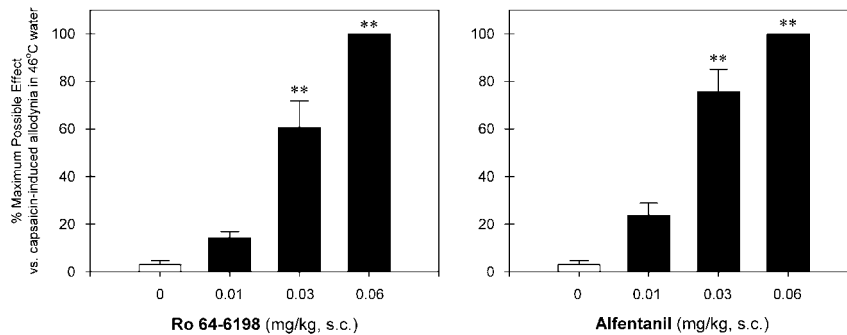
**Figure 2** Effects of  $\mu$ -opioid receptor and NOP receptor antagonists on alfentanil- and Ro 64-6198-induced antinociceptive effects in monkeys. A  $\mu$ -opioid receptor antagonist (naltrexone, 0.03 mg/kg, s.c.) or an NOP receptor antagonist (J-113397, 0.1 mg/kg, s.c.) was administered s.c. 15 min before redetermination of the dose-response curve of alfentanil and Ro 64-6198. Left panel, antagonist effects of s.c. naltrexone and J-113397 on the dose-response curve of Ro 64-6198-induced antinociception in 50°C water. Right panel, antagonist effects of s.c. naltrexone and J-113397 on the dose-response curve of alfentanil-induced antinociception in 50°C water. Each data point represents a mean  $\pm$  SEM ( $n = 6$ ).

Mean  $ED_{50}$  (95% confidence limit) value of s.c. Ro 64-6198-induced antinociception with vehicle pretreatment was 0.014 mg/kg (0.011–0.016). Pretreatment with J-113397 dose-dependently produced rightward shifts of the dose-response curve of Ro 64-6198-induced antinociception. These dose-dependent antagonist effects of J-113397 were graphed in a Schild plot with values derived from individual dose ratios for each subject. The mean  $pA_2$  value of J-113397 was 7.98 (7.85–8.11) with a slope of  $-1$ . The doses of J-113397 alone did not change the thermal threshold of monkeys (ie, no changes in the tail-withdrawal latencies in 42, 46, or 50°C water).

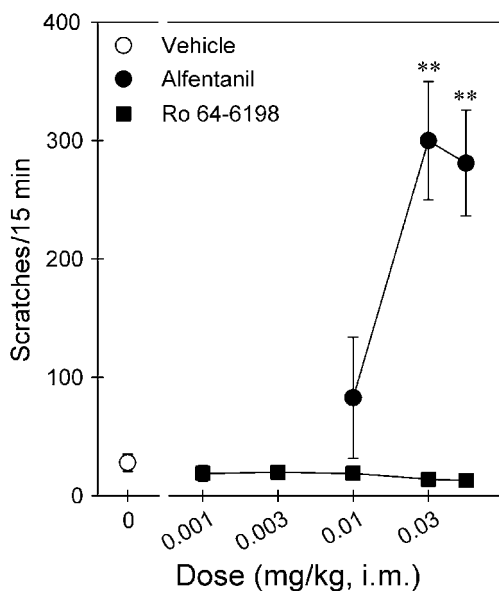
Figure 2 compares the antagonist effects of naltrexone and J-113397 on the antinociceptive effects produced by s.c. Ro 64-6198 and alfentanil. The left panel shows that a single dose (0.1 mg/kg) of J-113397 produced a large rightward shift of the dose-response curve of Ro 64-6198-induced antinociception. The mean J-113397  $pK_B$  value was 8.02

(7.78–8.26) under this condition. Naltrexone 0.03 mg/kg failed to block Ro 64-6198-induced antinociception; the  $ED_{50}$  value of Ro 64-6198 dose-response for vehicle pretreatment (0.012 mg/kg) was similar to that for naltrexone pretreatment (0.013 mg/kg). In contrast, the right panel shows that a single dose of naltrexone 0.03 mg/kg produced a large rightward shift of the dose-response curve of alfentanil-induced antinociception. The mean naltrexone  $pK_B$  value was 8.44 (8.18–8.70) under this condition. J-113397 0.1 mg/kg failed to block alfentanil-induced antinociception; the  $ED_{50}$  value of alfentanil dose-response for vehicle pretreatment (0.031 mg/kg) was similar to that for J-113397 pretreatment (0.026 mg/kg).

Figure 3 illustrates the antinociceptive effects of Ro 64-6198 and alfentanil against capsaicin-induced allodynia. Normally, monkeys kept their tails in 46°C water for 20 s, but withdrew their tails within 1–3 s after capsaicin injection (mean  $\pm$  SEM,  $1.7 \pm 0.2$  s). Pretreatment with Ro 64-6198



**Figure 3** Antinociceptive effects of Ro 64-6198 and alfentanil against capsaicin-induced allodynia in 46°C water. Each data point represents a mean  $\pm$  SEM ( $n = 6$ ). The asterisks represent a significant difference from the vehicle condition (\*\* $p < 0.01$ ). Each data point was measured at 15 min after administration of capsaicin.



**Figure 4** Comparison of the dose-response curves for itch/scratching effects produced by i.m. administration of alfentanil and Ro 64-6198. Each data point represents a mean  $\pm$  SEM ( $n = 6$ ). The asterisks represent a significant difference from the vehicle condition (\*\* $p < 0.01$ ).

( $F(3,20) = 60.6$ ;  $p < 0.01$ ) and alfentanil ( $F(3,20) = 68.3$ ;  $p < 0.01$ ) both dose-dependently attenuated allodynia in 46°C water. The  $ED_{50}$  value for Ro 64-6198 dose-response (0.024 mg/kg) was similar to that for alfentanil (0.019 mg/kg) under this condition.

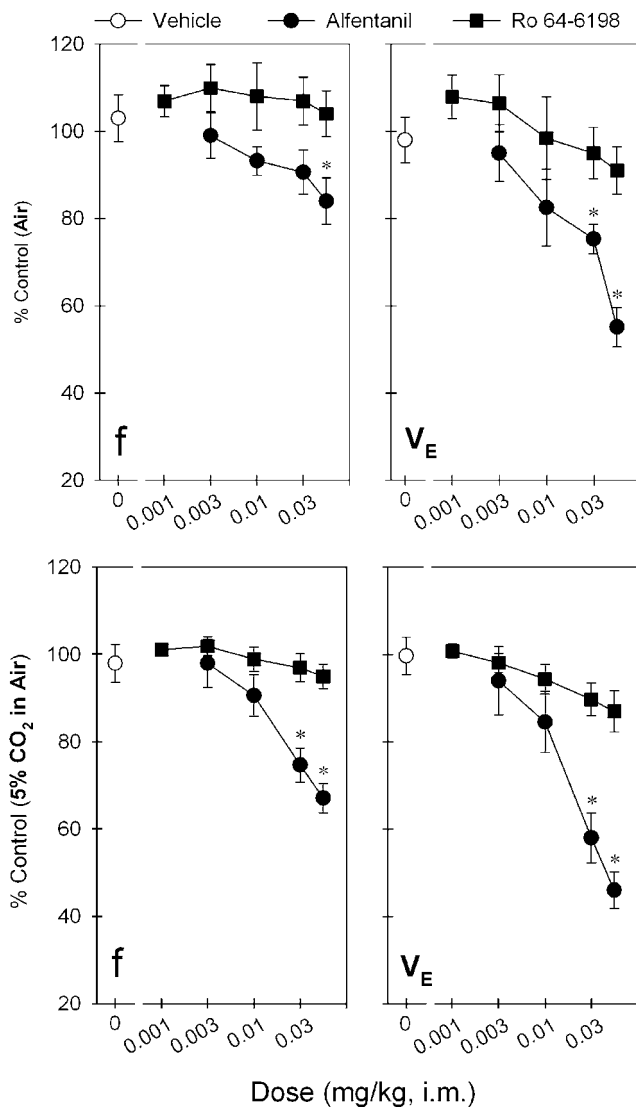
Figure 4 compares the itch/scratching responses of alfentanil and Ro 64-6198 after i.m. administration. Alfentanil produced a dose-dependent increase in scratching ( $F(3,20) = 11.0$ ;  $p < 0.05$ ). *Post hoc* comparisons indicated that both doses of alfentanil 0.03 and 0.06 mg/kg significantly increased scratching responses ( $p < 0.01$ ). The peak effect was  $300 \pm 49.9$  (mean  $\pm$  SEM) scratches evoked by 0.03 mg/kg of alfentanil. In contrast, Ro 64-6198 did not increase scratching responses ( $F(5,30) = 0.7$ ;  $p > 0.05$ ), compared with the vehicle condition in the same monkeys. These doses of Ro 64-6198 (ie, 0.001–0.06 mg/kg) did not produce any observable sedation in monkeys.

Figure 5 compares the respiratory depressant effects of alfentanil and Ro 64-6198 after i.m. administration. The top panels show the dose-response curves of alfentanil and Ro 64-6198 for the changes of respiratory parameters  $f$  and  $V_E$  during air breathing. Alfentanil produced dose-dependent changes for both  $f$  ( $F(4,25) = 3.3$ ;  $p < 0.05$ ) and  $V_E$  ( $F(4,25) = 9.3$ ;  $p < 0.05$ ). *Post hoc* comparisons indicated that alfentanil 0.06 mg/kg significantly decreased  $f$  responses ( $p < 0.05$ ). In addition, both doses of alfentanil, 0.03 and 0.06 mg/kg, significantly decreased  $V_E$  responses ( $p < 0.05$ ). The maximum depressant effect of  $V_E$  responses produced by alfentanil 0.06 mg/kg was  $55 \pm 5\%$  of control response (ie, before drug administration). In contrast, Ro 64-6198 did not decrease the respiratory function manifested by  $f$  ( $F(5,30) = 0.2$ ;  $p > 0.05$ ) and  $V_E$  ( $F(5,30) = 1.4$ ;  $p > 0.05$ ) responses, compared with the vehicle condition in the same monkeys.

The bottom panels show the dose-response curves of alfentanil and Ro 64-6198 for the changes of respiratory parameters  $f$  and  $V_E$  during breathing of a mixture of 5%  $CO_2$  in air. This increase in  $CO_2$  enhances the sensitivity of the assay to the potential respiratory depressant effects of test compounds. Alfentanil produced dose-dependent changes of both  $f$  ( $F(4,25) = 14.1$ ;  $p < 0.05$ ) and  $V_E$  ( $F(4,25) = 19.4$ ;  $p < 0.05$ ) under these conditions. *Post hoc* comparisons indicated that both alfentanil 0.03 and 0.06 mg/kg significantly decreased  $f$  and  $V_E$  responses ( $p < 0.05$ ). The maximum respiratory depressant effect produced by alfentanil 0.06 mg/kg was  $67 \pm 3$  and  $46 \pm 4\%$  of control  $f$  and  $V_E$  responses, respectively. In contrast, Ro 64-6198 did not significantly decrease the respiratory parameters  $f$  ( $F(5,30) = 1.3$ ;  $p > 0.05$ ) and  $V_E$  ( $F(5,30) = 2.4$ ;  $p > 0.05$ ), compared with the vehicle condition in the same monkeys.

Figure 6 top panel shows the reinforcing effects of Ro 64-6198 in alfentanil-maintained monkeys. Response rates (responses/s) for saline, alfentanil, and Ro 64-6198 across a dose range of 0.03–30  $\mu$ g/kg per injection were assessed. To aggregate data across all three subjects, mean response rates engendered by each dose of each drug were averaged. Under the multiple component schedules, contingent saline infusions engendered very low response rates ( $< 0.3$  responses/s). The top panel of Figure 6 presents the aggregate dose-response curves for alfentanil and Ro 64-6198. All animals self-administered alfentanil within the

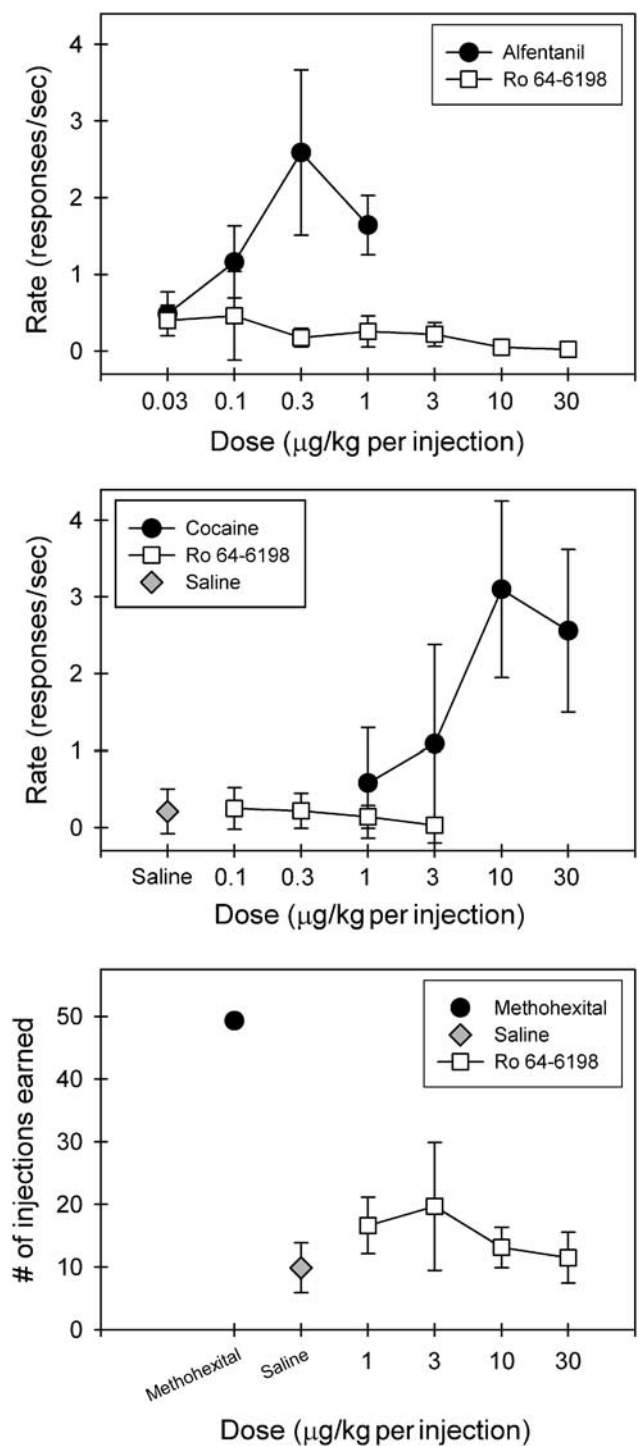




**Figure 5** Comparison of the dose-response curves for respiratory depressant effects produced by i.m. administration of alfentanil and Ro 64-6198. Top and bottom panels show the changes of both  $f$  and  $V_E$  parameters when monkeys breathing air and air mixed with 5%  $\text{CO}_2$ , respectively. Each data point represents a mean  $\pm$  SEM ( $n=6$ ). The asterisk represent a significant difference from the vehicle condition ( $*p<0.05$ ).

dose range tested, generating a biphasic dose-effect curve characteristic of i.v. drug self-administration. In contrast, Ro 64-6198 did not maintain high rates of responding at any of the doses tested, resulting in a flat dose-effect curve indicative of a compound without reinforcing effects under the present conditions. Likewise, the middle panel indicates that Ro 64-6198 did not maintain high rates of responding at the doses tested, although all subjects self-administered cocaine under the same schedule.

Figure 6 bottom panel presents the aggregate dose-response curves for Ro 64-6198 compared with responding maintained by a reference dose of methohexital or saline. The number of injections earned of Ro 64-6198 across a dose range of 1–30  $\mu\text{g/kg}$  per injection were compared to the number of self-injections earned of 0.1 mg per kg/injection methohexital or saline. To aggregate data across all three



**Figure 6** Lack of reinforcing effects of Ro 64-6198 in alfentanil-, cocaine-, and methohexital-maintained monkeys. Top and middle panels: Symbols represent aggregated dose-response curves for alfentanil, cocaine, and Ro 64-6198 under a fixed ratio of 30 timeout 45 s multiple component schedule. Data are the means  $\pm$  SEM ( $n=3$ ) for the response rates. Bottom panel: symbols represent aggregated dose-response curves for Ro 64-6198 compared with responding maintained by a single dose of methohexital 0.1 mg/kg/injection or saline. Data are the means  $\pm$  SEM ( $n=3$ ) for the numbers of injection earned.

experimental animals, mean number of injections earned by each monkey at each dose were averaged. Methohexital-maintained responding occurred at a high, regular rate

across the entire session. When contingent saline was available, animals tended to 'sample' early in the session, but behavior generally abated entirely within 15 min. No dose of Ro 64-6198 reliably maintained responding above levels observed when saline was available, indicating that Ro 64-6198 had no reinforcing effects under the present conditions.

## DISCUSSION

Systemic Ro 64-6198 alone produced antinociceptive effects that were blocked dose-dependently by J-113397, a selective NOP receptor antagonist. *In vivo* apparent  $pA_2$  analysis was used because this quantitative procedure offers a powerful approach to establish receptor-mediated drug effects (Arunlakshana and Schild, 1959; Tallarida *et al*, 1979). In this study, J-113397 dose-dependently produced parallel rightward shifts of the dose-response curve of Ro 64-6198-induced antinociception (Figure 1), indicating that the agonist and antagonist compete for the same NOP receptors in a reversible manner. The  $pA_2$  value of J-113397, 8.0, was approximately threefold less than the naltrexone  $pA_2$  value of 8.5 under the same behavioral context using an antinociceptive assay (Ko *et al*, 1998a), indicating that both naltrexone and J-113397 are potent antagonists *in vivo* for  $\mu$ -opioid and NOP receptors, respectively, in monkeys. More important, examination of both antagonists against different agonists showed that alfentanil- and Ro 64-6198-induced antinociceptive effects were mediated by  $\mu$ -opioid receptors and NOP receptors, respectively (Figure 2). J-113397 0.1 mg/kg failed to block alfentanil-induced antinociception and naltrexone 0.03 mg/kg failed to block Ro 64-6198-induced antinociception. These results indicate that antinociceptive effects of opioid analgesics are produced by two independent opioid receptor mechanisms in monkeys.

Systemic administration of Ro 64-6198-produced antinociception against capsaicin-induced allodynia in monkeys (Figure 3). Capsaicin evokes pain sensation by activating at the vanilloid receptor and stimulating the release of pronociceptive neuropeptides, such as substance P from primary afferents (Szallasi *et al*, 2007). Studies have shown that the vanilloid receptor is required for inflammatory sensitization to noxious stimuli and is essential for tissue injury-induced allodynia and hyperalgesia (Caterina *et al*, 2000; Davis *et al*, 2000). Capsaicin-induced allodynia has been used in both monkeys (Ko *et al*, 1998b; Butelman *et al*, 2004) and humans (Park *et al*, 1995; Eisenach *et al*, 1997) to show its prominent value for studying pain mechanisms *in vivo* and pharmacological interventions. Given that capsaicin-sensitive nerve fibers are involved in a variety of nociceptive conditions (Szallasi *et al*, 2007), the effectiveness of Ro 64-6198 in inhibiting capsaicin-induced allodynia indicates that NOP receptor agonists may be effective for treating pain derived from different nociceptive origins.

It is worth noting that systemic Ro 64-6198 did not produce antinociceptive effects in rodents (Jenck *et al*, 2000). Perhaps supraspinal NOP receptor-mediated hyperalgesia in rodents (Meunier *et al*, 1995; Rizzi *et al*, 2007) counteract antinociceptive effects mediated by spinal and

peripheral NOP receptors when rodents receive systemic administration of non-peptidic NOP receptor agonists. Given that both systemic and spinal administration routes are commonly used for delivery of analgesics in humans, it may not be practical to study the effects of intracerebroventricular administration of NOP receptor agonists in monkeys. Nevertheless, the degree of integrated physiological outcome from activating supraspinal, spinal, and peripheral NOP receptors together following systemic administration of NOP receptor agonists may vary across species. Anatomical studies have indicated that differences between rodents and primates may exist in the distribution of N/OFQ and NOP receptors (Berthele *et al*, 2003; Bridge *et al*, 2003). In addition, functional studies have also revealed that species differences exist in the pharmacological profiles of spinal N/OFQ between rodents and primates (Inoue *et al*, 1999; Sakurada *et al*, 1999). Unlike dual actions (ie, both pronociceptive and antinociceptive effects) of intrathecal N/OFQ observed in rodents, intrathecal N/OFQ only produced antinociceptive effects in monkeys (Ko and Naughton, 2009). More research should be conducted to elucidate whether the signal transduction pathways of NOP receptors or/and functions of sensory neurons expressing NOP receptors are different between rodents and primates.

The antinociceptive doses of systemic Ro 64-6198 (ie, 0.01–0.06 mg/kg) did not produce undesirable side effects compared with the  $\mu$ -opioid agonist alfentanil (Figures 3 and 4). Both respiratory depression and itch/scratching have been documented as physiological responses to  $\mu$ -opioid receptor activation in monkeys (Butelman *et al*, 1993; Ko *et al*, 2004). Given that these doses of Ro 64-6198 did not produce any sedation or motor dysfunction in monkeys, systemic Ro 64-6198 provides a promising pharmacological profile of NOP receptors as a novel analgesic in primates. On the other hand, rodent studies have found that higher doses of systemic Ro 64-6198 (10 mg/kg) interfered with behavioral performance (Jenck *et al*, 2000; Shoblock, 2007). These results suggest that Ro 64-6198 may have a wide therapeutic window between the antinociceptive doses and doses eliciting undesirable side effects. Whereas this study suggests that Ro 64-6198 may have a wide therapeutic index relative to the  $\mu$ -opioid agonist alfentanil, it does not establish what the dose-limiting effects of this compound might be. Administration of larger doses of Ro 64-6198 and other systemically active NOP receptor agonists are needed to establish dose-limiting effects.

No reinforcing effects of Ro 64-6198 in alfentanil-, cocaine-, and methohexital-maintained monkeys (Figure 6) were observed. The presence of a behavioral effect (ie, antinociception at 10–30  $\mu$ g/kg) in the absence of any indication of a reinforcing effect indicates that we have tested sufficiently large doses for potential reinforcing effects. For example, the antinociceptive doses of i.v. alfentanil were 10–30  $\mu$ g/kg (Ko *et al*, 2002a), but the doses of alfentanil-producing reinforcing effects were 0.1–1  $\mu$ g/kg (ie, a 30–100-fold difference; Winger *et al*, 1992; Ko *et al*, 2002a). Lack of reinforcing effects by Ro 64-6198 might be expected because several studies have shown that the activation of NOP receptors inhibited dopamine release in the striatum, and supported the notion that NOP receptor

agonists do not have reinforcing or aversive properties of their own (Murphy and Maidment, 1999; Flau *et al*, 2002). Given that increased dopamine neuronal activity is closely associated with reinforcing effects of several drugs of abuse, it will be valuable to study further whether NOP receptor agonists can suppress the reinforcing effects of other drugs that have abuse potential in primates.

Taken together, this study showed that antinociceptive effects of systemic Ro 64-6198 were independent of  $\mu$ -opioid receptors and activation of NOP receptors produced antinociception without reinforcing effects in monkeys. Ro 64-6198 has previously been studied in only rodent species (Chiou *et al*, 2007; Shoblock, 2007). This is the first study to investigate the behavioral effects of Ro 64-6198 in primates. Like alfentanil, Ro 64-6198 produced antinociception in two primate nociceptive models. Unlike alfentanil, Ro 64-6198 did not produce reinforcing effects, respiratory depressant, or itch/pruritic side effects, indicating that NOP receptor agonists may be a new generation of novel analgesics without abuse liability. Such a promising pharmacological profile warrants additional studies to document potential therapeutic value of NOP receptor agonists in humans.

## ACKNOWLEDGEMENTS

We thank Dr Gail Winger for her assistance with the editing of manuscript and John Busenbark, Tristan Edwards, and Wayne Yang for excellent technical assistance. **This study was supported by the US Department of Defense, Peer Reviewed Medical Research Program, Grant W81XWH-07-1-0162 to Mei-Chuan Ko and the US Public Health Service Grant DA-015449 to Gail Winger.**

## DISCLOSURE/CONFLICT OF INTEREST

The authors declare that, except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service except as listed below, and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest. The author MCK received research support from Grunenthal GmbH, Ingenium Pharmaceuticals, Amgen, SSV Therapeutics, and Elan Pharmaceuticals. The author JHW received research support from Grunenthal GmbH, Alkermes, Roche, Adolor Corporation, and Reckitt Benckiser Pharmaceuticals.

## REFERENCES

- Arunlakshana O, Schild HO (1959). Some quantitative uses of drug antagonists. *Br J Pharmacol* 14: 48–58.
- Ator NA, Griffiths RR (1987). Self-administration of barbiturates and benzodiazepines: a review. *Pharmacol Biochem Behav* 27: 391–398.
- Berthele A, Platzer S, Dworak D, Schadrack J, Mahal B, Butter A *et al* (2003). [3H]-Nociceptin ligand-binding and nociception opioid receptor mRNA expression in the human brain. *Neuroscience* 121: 629–640.
- Bridge KE, Wainwright A, Reilly K, Oliver KR (2003). Autoradiographic localization of 125I[Tyr14]nociceptin/orphanin FQ

- binding sites in macaque primate CNS. *Neuroscience* 118: 513–523.
- Butelman ER, France CP, Woods JH (1993). Apparent  $pA_2$  analysis on the respiratory depressant effects of alfentanil, etonitazene, ethylketocyclazocine(EKC) and Mr2033 in rhesus monkeys. *J Pharmacol Exp Ther* 264: 145–151.
- Butelman ER, Harris TJ, Kreek MJ (2004). Antiallodynic effects of loperamide and fentanyl against topical capsaicin-induced allodynia in unanesthetized primates. *J Pharmacol Exp Ther* 311: 155–163.
- Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR *et al* (2000). Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 288: 306–313.
- Chiou LC, Liao YY, Fan PC, Kuo PH, Wang CH, Riemer C *et al* (2007). Nociceptin/orphanin FQ peptide receptors: pharmacology and clinical implications. *Curr Drug Targets* 8: 117–135.
- Cicero TJ, Dart RC, Inciardi JA, Woody GE, Schnoll S, Munoz A (2007). The development of a comprehensive risk-management program for prescription opioid analgesics: researched abuse, diversion and addiction-related surveillance (RADARS). *Pain Med* 8: 157–170.
- Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P *et al* (2000). Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature* 405: 183–187.
- Dykstra LA, Gmerek DE, Winger G, Woods JH (1987). Kappa opioids in rhesus monkeys. I. Diuresis, sedation, analgesia and discriminative stimulus effects. *J Pharmacol Exp Ther* 242: 413–420.
- Eisenach JC, Hood DD, Curry R, Tong C (1997). Alfentanil, but not amitriptyline, reduces pain, hyperalgesia, and allodynia from intradermal injection of capsaicin in humans. *Anesthesiology* 86: 1279–1287.
- Erb K, Liebel JT, Tegeder I, Zeilhofer HU, Brune K, Geisslinger G (1997). Spinally delivered nociceptin/orphanin FQ reduces flinching behaviour in the rat formalin test. *Neuroreport* 8: 1967–1970.
- Flau K, Redmer A, Liedtke S, Kathmann M, Schlicker E (2002). Inhibition of striatal and retinal dopamine release via nociceptin/orphanin FQ receptors. *Br J Pharmacol* 137: 1355–1361.
- Foord SM, Bonner TI, Neubig RR, Rosser EM, Pin J-P, Davenport AP *et al* (2005). International Union of Pharmacology. XLVI. G Protein-Coupled Receptor List. *Pharmacol Rev* 57: 279–288.
- Inoue M, Shimohira I, Yoshida A, Zimmer A, Takeshima H, Sakurada T *et al* (1999). Dose-related opposite modulation by nociceptin/orphanin FQ of substance P nociception in the nociceptors and spinal cord. *J Pharmacol Exp Ther* 291: 308–313.
- Jenck F, Wichmann J, Dautzenberg FM, Moreau JL, Ouagazzal AM, Martin JR *et al* (2000). A synthetic agonist at the orphanin FQ/nociceptin receptor ORL1: Anxiolytic profile in the rat. *Proc Natl Acad Sci USA* 97: 4938–4943.
- Katz NP, Adams EH, Benneyan JC, Birnbaum HG, Budman SH, Buzzeo RW *et al* (2007). Foundations of opioid risk management. *Clin J Pain* 23: 103–118.
- Kawamoto H, Ozaki S, Itoh Y, Miyaji M, Arai S, Nakashima H *et al* (1999). Discovery of the first potent and selective small molecule opioid receptor-like (ORL1) antagonist: 1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1, 3-dihydro-2H-benzimidazol-2-one (J-113397). *J Med Chem* 42: 5061–5063.
- Ko MC, Butelman ER, Traynor JR, Woods JH (1998a). Differentiation of kappa opioid agonist-induced antinociception by naltrexone apparent  $pA_2$  analysis in rhesus monkeys. *J Pharmacol Exp Ther* 285: 518–526.
- Ko MC, Butelman ER, Woods JH (1998b). The role of peripheral mu opioid receptors in the modulation of capsaicin-induced thermal nociception in rhesus monkeys. *J Pharmacol Exp Ther* 286: 150–156.

- Ko MC, Naughton NN (2009). Antinociceptive effects of nociception/orphanin FQ administered intrathecally in monkeys. *J Pain* (in press) doi: 10.1016/j.pain.2008.11.006.
- Ko MC, Terner J, Hursh S, Woods JH, Winger G (2002a). Relative reinforcing effects of three opioids with different durations of action. *J Pharmacol Exp Ther* 301: 698–704.
- Ko MCH, Naughton NN, Traynor JR, Song MS, Woods JH, Rice KC et al (2002b). Orphanin FQ inhibits capsaicin-induced thermal nociception in monkeys by activation of peripheral ORL1 receptors. *Br J Pharmacol* 135: 943–950.
- Ko MCH, Song MS, Edwards T, Lee H, Naughton NN (2004). The role of central mu opioid receptors in opioid-induced itch in primates. *J Pharmacol Exp Ther* 310: 169–176.
- Ko MCH, Wei H, Woods JH, Kennedy RT (2006). Effects of intrathecally administered nociceptin/orphanin FQ in monkeys: Behavioral and mass spectrometric studies. *J Pharmacol Exp Ther* 318: 1257–1264.
- Kuhar MJ, Pert CB, Snyder SH (1973). Regional distribution of opiate receptor binding in monkey and human brain. *Nature* 245: 447–450.
- Mansour A, Khachaturian H, Lewis ME, Akil H, Watson SJ (1988). Anatomy of CNS opioid receptors. *Tr Neurosci* 11: 308–314.
- Meunier JC, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P et al (1995). Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. *Nature* 377: 532–535.
- Mollereau C, Parmentier M, Mailleux P, Butour JL, Moisand C, Chalon P et al (1994). ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. *FEBS Lett* 341: 33–38.
- Murphy NP, Maidment NT (1999). Orphanin FQ/nociceptin modulation of mesolimbic dopamine transmission determined by microdialysis. *J Neurochem* 73: 179–186.
- Negus SS, Gatch MB, Mello NK, Zhang X, Rice K (1998). Behavioral effects of the delta-selective opioid agonist SNC80 and related compounds in rhesus monkeys. *J Pharmacol Exp Ther* 286: 362–375.
- Obara I, Przewlocki R, Przewlocka B (2005). Spinal and local peripheral antiallodynic activity of Ro 64-6198 in neuropathic pain in the rat. *Pain* 116: 17–25.
- Park KM, Max MB, Robinovitz E, Gracely RH, Bennett GJ (1995). Effects of intravenous ketamine, alfentanil, or placebo on pain, pinprick hyperalgesia, and allodynia produced by intradermal capsaicin in human subjects. *Pain* 63: 163–172.
- Peckys D, Landwehrmeyer GB (1999). Expression of mu, kappa, and delta opioid receptor messenger RNA in the human CNS: a 33P *in situ* hybridization study. *Neuroscience* 88: 1093–1135.
- Reinscheid RK, Nothacker HP, Bourson A, Ardati A, Henningsen RA, Bunzow JR et al (1995). Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. *Science* 270: 792–794.
- Rizzi A, Spagnolo B, Wainford RD, Fischetti C, Guerrini R, Marzola G et al (2007). *In vitro* and *in vivo* studies on UFP-112, a novel potent and long lasting agonist selective for the nociceptin/orphanin FQ receptor. *Peptides* 28: 1240–1251.
- Sakurada T, Katsuyama S, Sakurada S, Inoue M, Tan-No K, Kisara K et al (1999). Nociceptin-induced scratching, biting, and licking in mice: involvement of spinal NK1 receptors. *Br J Pharmacol* 127: 1712–1718.
- Shoblock JR (2007). The pharmacology of Ro 64-6198, a systemically active, nonpeptide NOP receptor (opiate receptor-like 1, ORL1) agonist with diverse preclinical therapeutic activity. *CNS Drug Rev* 13: 107–136.
- Szallasi A, Cortright DN, Blum CA, Eid SR (2007). The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof of concept. *Nat Rev Drug Discov* 6: 357–372.
- Tallarida RJ, Cowan A, Adler MW (1979). pA2 and receptor differentiation: a statistical analysis of competitive antagonism. *Life Sci* 25: 637–654.
- Weerts EM, Fantegrossi WE, Goodwin AK (2007). The value of nonhuman primates in drug abuse research. *Exp Clin Psychopharmacol* 15: 309–327.
- Wichmann J, Adam G, Rover S, Hennig M, Scalone M, Cesura AM et al (2000). Synthesis of (1S,3aS)-8-(2,3,3a,4,5, 6-hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4. 5]decan-4-one, a potent and selective orphanin FQ (OFQ) receptor agonist with anxiolytic-like properties. *Eur J Med Chem* 35: 839–851.
- Winger G, Skjoldager P, Woods JH (1992). Effects of buprenorphine and other opioid agonists and antagonists on alfentanil- and cocaine-reinforced responding in rhesus monkeys. *J Pharmacol Exp Ther* 261: 311–317.
- Winger G, Stitzer ML, Woods JH (1975). Barbiturate-reinforced responding in rhesus monkeys: comparisons of drugs with different durations of action. *J Pharmacol Exp Ther* 195: 505–514.
- Zeilhofer HU, Calo G (2003). Nociceptin/orphanin FQ and its receptor—potential targets for pain therapy? *J Pharmacol Exp Ther* 306: 423–429.
- Zollner C, Stein C (2007). Opioids. *Handb Exp Pharmacol* 177: 31–63.